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TOXICITY OF METHOXYCHLOR TO FISH

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TOXICITY OF METHOXYCHLOR TO FISH

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SUMMARY

The objective of this study was to determine the acute, subacute and chronic effects of methoxychlor to fish, principally rainbow trout.

The acute 96-h LC_{50} of methoxychlor (in µg/L as methoxychlor) was 9.4-31 for rainbow trout, 20 for walleye, 24 for spottail shiner, >31 for yellow perch, 260 for white sucker, and 4,700 for fathead minnow. Tolerance to the pesticide increased as the duration of exposure decreased. The emulsifying agent used in the industrial preparation of the pesticide (Sanex-methoxychlor 25E) was found to be 3 orders of magnitude less toxic to rainbow trout than methoxychlor. In emulsified mixtures of the pesticide, the emulsifier and methoxychlor interacted to produce simple additive or perhaps less-than-additive toxic effects.

Sanex-methoxychlor 25E was not acutely toxic to early life stages of fish. No age-dependent effects were found in young fathead minnows; the 96-h LC_{50} of Sanex-methoxychlor 25E ranged from 1.8 to 2.9 µg/L regardless of fish age between 1 and 7 weeks. Concentrations of $\leq$580 µg/L methoxychlor had no statistically significant erfect on hatching behaviour or hatching success of rainbow trout, when the fish were exposed to a 2-h pulse as eggs approximately one week prehatch.

Exposure of freshly fertilized rainbow trout eggs (1.5 d post-fertilization) to spikes of methoxychlor at nominal concentrations of $\leq$30 µg/L had no detectable effect on yolk absorption, fish growth, egg hatchability or subsequent hatchling (alevin) survival. At 30 µg/L, however, spiked exposure of freshly fertilized eggs retarded hatching.

Rainbow trout were also exposed to methoxychlor (reagent-grade and industrial-grade), the emulsifier and a solvent (ethanol) for up to 28 d to determine possible histopathological effects in selected tissues. Methoxychlor (both grades) was applied at concentrations of up to 40 µg/L in the experimental tanks, whereas the application dose of the emulsifier ranged from 30 to 120 µg/L. Fish were sampled at the beginning of the experiments and at day 7, 14, 21 and 28. Tissues examined by light microscopy were gills, heart, oral cavity, eye, brain, spinal cord, liver, stomach, pyloric ceca, intestine, pancreas, spleen, anterior and posterior kidneys, gonad, skin and muscle. Livers were also examined using transmission electron microscopy. Lesions were found only in gill and liver tissues. Lesions of the liver, primarily hepatic vacuolization, were not indicative of methoxychlor or emulsifier toxicity. Gill lesions (clubbing of lamellae) were also not indicative of methoxychlor or emulsifier toxicity. The reasons for the presence of lesions in gills and liver are unknown but may have been related to feeding conditions or water quality.

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PART I

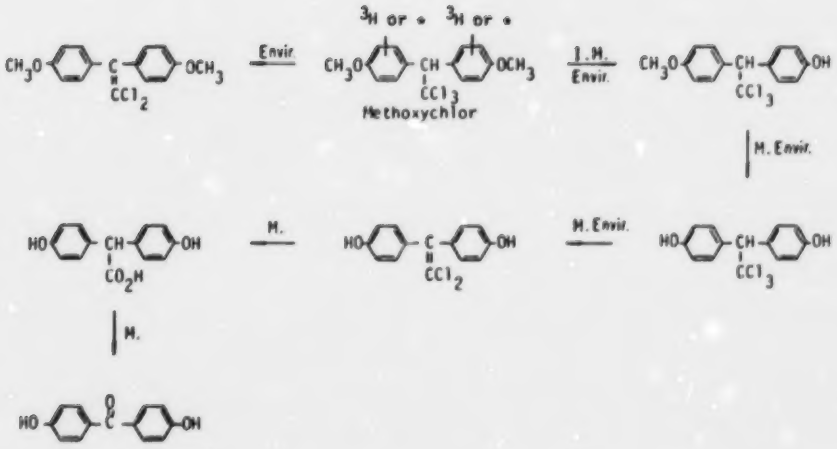
BACKGROUND

1. GENERAL INTRODUCTION

1.1 Background

Methoxychlor (MEO-DDT), the p,p'-methoxy analogue of DDT (Figure 1), is a broad-spectrum chlorinated hydrocarbon insecticide. It is used mainly to control biting fly larvae, particularly black flies and mosquitoes, in surface waters. Although methoxychlor also finds some application as a household and garden spray, the amount introduced to the environment through this route is probably minimal. Although methoxychlor may partition favourably in fatty deposits in target and non-target organisms, other tissues such as liver and kidney may also contain appreciable amounts of methoxychlor (Lockhart et al., 1977; AEC, 1984).

In the natural environment, methoxychlor may undergo dehydrochlorination to form a number of products (Figure 1). Complete hydrolysis of the methyl ether bonds has also been reported. Most animals are capable of metabolizing methoxychlor through dealkylation and dehydrochlorination. The resulting products are generally water soluble and readily eliminated through urine and faeces. Although 2, 2-bis(p-methoxyphenyl)-1, 1-dichloroethylene (MEO-DDE) is a major metabolite, other compounds such as 1-hydroxyphenyl-2-methoxyphenyl-1-chloro-2-dichloroethane, 1-hydroxyphenyl-2-methoxyphenyl-1,2-dichloroethylene and polar metabolites may also be produced (NRCC, 1975). Since these compounds are rapidly eliminated, they are generally not found in tissues (NRCC, 1975).



Mammal	Female Swiss mice
Insect	Female SP (DDT resistant) housefly; Fifth instar larvae of salt marsh caterpillar: *Estigmene acrea*
Environment	A model ecosystem = Plant: *Sorghum halpense;* Algae: *Oedogonium cardiacum;* Snails: *Daphnia magna, Physa;* Larvae of the salt marsh caterpillar: *Estigmene acrea;* Mosquito larvae: *Culex quinguefasciatus;* Fish: *Gambusia affinis*

Figure 1. Breakdown products of methoxychlor. SOURCE: Aizawa (1982).

Methoxychlor is one of the few chlorinated pesticides that continues to find use in western nations. Methoxychlor is not nearly as persistent as DDT, and accumulates at a much slower rate than DDT (NRCC, 1975). Accordingly, impact on non-target organisms is typically minimal compared to that by DDT.

In Alberta, the main environmental interest surrounding the use of methoxychlor is its application to the Athabasca River. Control of black fly larvae in the vicinity of the Town of Athabasca is considered important because of effects on livestock. The river has been treated for more than a decade. Typically, 2 or 3 doses are applied each spring/summer at rates of 0.275-0.3 mg/L. There have already been a number of studies on methoxychlor and its impact on the Athabasca. Some of the major findings are listed here.

a. A single 0.3 mg/L injection for 15 minutes caused a catastrophic drift of aquatic invertebrates over 400 km of the Athabasca River (Flannagan et al., 1979).

b. No mortalities were observed for caged crayfish or clams following treatment.

c. Invertebrate populations in the substrate typically decline following treatment at 0.3 mg/L. This effect may be noted more than 200 km downstream of the treatment site for some species (Byrtus, 1981a, 1982b, 1982; Byrtus and Jackson, 1984, 1986; Murray, 1983; Murray and Jackson, 1982). Other species may remain largely unaffected during the treatment.

d. No mortality has been observed in fish (Haufe, 1980).

e. Fish caged in the Athabasca River sorbed methoxychlor rapidly when exposed to a nominal dose of 0.3 mg/L (Lockhart et al., 1977). Residues declined to below the detection limit within two weeks of exposure.

f. Fish samples, collected 14 days after treatment at 0.3 mg/L, contained no detectable concentration of methoxychlor or a breakdown product 2,2-bis(p-methoxyphenyl)-1,1-dichloroethylene (AEC, 1984).

g. Methoxychlor residues >0.1 µg/L (ppb) (limit of detection) have been found in river water more than 200 km downstream of the Town of Athabasca (Byrtus and Jackson, 1984, 1986).

1.2 Objective of Study

The objective of this study was to determine the acute, subacute and chronic effects of methoxychlor to fish, principally rainbow trout.

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PART II

DETERMINATION OF METHOXYCHLOR RESIDUES IN WATER, SEDIMENT AND FISH SAMPLES

1. INTRODUCTION

1.1 General

The methods outlined here were used to determine methoxychlor residues in water, sediment and fish samples from the major subprojects of this study. Water samples of 1 L were collected in precleaned 1-L amber glass bottles. Approximately 500 g of sediment samples were collected in precleaned amber glass ointment jars. The samples were transferred to the Chemistry Division Pesticide Analysis Laboratory for residue analysis as soon as possible after they were collected. The samples were stored in a refrigerator at 4°C until analyzed.

Fish were collected and dissected by the Aquatic Biology Branch staff. Samples of muscle, liver, gonad, kidney and fat tissues were collected and wrapped in aluminum foil. Samples were stored at -20°C until analysis.

1.2 Water Samples

Sample bottles were removed from the refrigerator and allowed to equilibrate to room temperature. The sample was spiked with Aldrin as a surrogate standard to monitor any losses that might take place through sample handling, and to reduce the possibility of false negative results. The samples were extracted with three serial 50-mL portions of dichloromethane in a 2-L separatory funnel. The extract was filtered through anhydrous sodium sulfate and concentrated to approximately 1 mL on a rotary evaporator. A gentle stream of

nitrogen was used to remove the remaining dichloromethane. The final extract was made up to 2 mL using 20% ethyl ether in hexane. This extract was transferred to an injection vial and sealed.

Analysis of this extract was carried out on a Varian 6000 gas chromatograph equipped with a 30-m fused silica DB-5 capillary column and an electron capture detector. Quantitation of the methoxychlor residues was achieved using an external standard method by comparing peak areas in sample chromatograms to the standard methoxychlor chromatograms. The method detection limit using that analytical procedure and analyzing spiked samples for percent recovery to fall within 99% confidence intervals was 0.03 µg/L. The accuracy of the method was 8.1%. The laboratory standard deviation, i.e. the precision, was 5.8%, and based on this, the repeatability of the method at 95% confidence was calculated to be 16.4. The absolute difference between two single test results will therefore be within 16.4, with 95% probability. The mean percent recovery of methoxychlor from spiked samples was 110.9 with a sample standard deviation of 5.4.

1.3 Sediment Samples

Sample jars were taken out of storage and allowed to equilibrate to room temperature. Sediment samples were air dried in a fume hood and extracted using a sonifier cell disruptor equipped with a microtip. The solvent of extraction was acetonitrile. Three serial 75 mL portions of acetonitrile were used to perform the extraction and all extracts were combined. This combined extract was transferred

to a 1-L separatory funnel and to it was added 25 mL of saturated sodium sulfate solution and 250 mL of organic free water. The separatory funnel was shaken vigorously for about 2 min. This solution was then extracted with three 75 mL portions of hexane and the extracts combined as before. The combined extracts were dried over anhydrous sodium sulfate and subsequently concentrated to about 1 mL on a rotary evaporator.

The extract was cleaned up using a column containing approximately 10 g of activated Florisil. The column was charged with the extract and eluted with 100 mL of hexane and the eluant discarded. The column was then eluted with 150 mL of 50% methylene chloride in hexane and that fraction was collected. The fraction was concentrated to near dryness using a rotary evaporator and a gentle stream of nitrogen. The final extract was made up to 1 mL using hexane. The instrumental analytical conditions for the analysis of the extract were similar to the water samples described earlier. The mean percent recovery of methoxychlor from spiked sediment samples was 104.1 with a sample standard deviation of 11.9. The method detection limit using the above analytical procedure and analyzing spiked samples, for percent recovery to fall within 99% confidence interval, was 0.002 µg/g.

1.4 Fish Samples

Fish samples were allowed to thaw at room temperature for 5 h prior to processing. Using a Polytron Homogenizer the samples were

ground to an homogeneous mixture. A 10-g sample of ground fish was transferred to an Erlenmeyer flask and to it was added 40 mL of Baker reagent-grade hydrochloric acid. The sample was allowed to stand overnight to digest the tissue. Approximately 25 mL of 25% methylene chloride in hexane was added to the flask and this sample was shaken on a wrist-shaker for 1 h. The contents of the Erlenmeyer flask were transferred to a 500-mL separatory funnel and the two layers were allowed to separate. The aqueous hydrochloric acid layer was collected and extracted once again on the wrist-shaker with 25 mL of the solvent mixture. The organic layers from the two extracts were combined and transferred to a 250-mL beaker, and the aqueous layer was then discarded. Approximately 2 g of sodium bicarbonate was used to neutralize the excess acid in the organic layer which was then dried over anhydrous sodium sulfate. The extract was then evaporated to approximately 1 mL on a rotary evaporator.

The extract was cleaned up using a Florisil column following the procedure described for sediments (Section 1.3). The final extract was made up to 1 mL using hexane.

The instrumental analytical conditions were identical to the water sample analyses described earlier (Section 1.2). The mean percent recovery of methoxychlor from spiked fish tissue samples was 81.8 with a sample standard deviation of 12.6. The method detection limit following this analytical procedure and analyzing a range of spiked samples for percent recovery to fall within 99% confidence interval, was 0.001 µg/g.

PART III

SPECTROSCOPIC CHARACTERIZATION OF THE HYDROCARBON CONTENT IN EMULSIFIER AND METHOXYCHLOR-ENRICHED EMULSIFIER

1. INTRODUCTION

Gas chromatography with flame ionization detection (GC/FID) and other spectroscopic techniques are widely used for fingerprinting hydrocarbon mixtures in gasoline (AEC, 1989; Hassan, 1981; Sanders and Maynard, 1981), petroleum (Cole, 1981), and fossil fuels (Plip, 1982), discussions of which are given in the literature (Warburton and Zumberge, 1982; Carter, 1982; Ury, 1981). In ideal cases, the GC/FID chromatogram can be used to characterize the source of oil spills (Cole, 1981; Carter, 1982), determine the extent of weathering a sample has undergone (AEC, 1989), and identify toxic components in hydrocarbon mixtures.

The degree of confidence in the GC/FID fingerprinting studies can, however, be hampered by several factors, some of which include (a) changes in the homogeneity of the sample, (b) chemical interference arising from non-hydrocarbons, and (c) discriminatory effects in the sample extraction procedures.

In this study, both GC/FID and combined gas chromatography mass spectrometry (GC/MS) were used to characterize the hydrocarbon content of the emulsifier and methoxychlor-enriched emulsifier samples. To minimize discriminatory effects during sample extraction, spectroscopic data were obtained directly for diluted samples using methylene chloride as the solvent. Likewise, preliminary studies were undertaken to study the effects of subsampling and homogeneity of the samples.

The work reported is qualitative, investigatory and appropriate for highlighting factors requiring further study.

2. MATERIALS AND METHODS

2.1 Sample Preparation Extraction

The relative solubility of the emulsifier in hexane, toluene and methylene chloride was observed. Aliquots of the emulsifier and methoxychlor-enriched emulsifier were either shaken or not shaken, diluted (2 µL/10 mL) using methylene chloride, and instrumental analyses performed using 2-µL injections.

2.2 Instrumental

2.2.1 Gas Chromatography/Flame Ionization Detection (GC/FID)

The analysis of the hydrocarbon content in the samples was performed using a Hewlett Packard (HP) 5840A gas chromatograph equipped with an HP 18850A integrator and a flame ionization detector. The gas chromatograph was also equipped with a fused silica DB-1 capillary column with length, internal diameter and film thickness of 30 m, 0.32 mm and 0.25 µm respectively. The carrier gas was hydrogen at a flow rate of 3.0 mL/min. The temperature of the column was programmed for 40°C for 1 min followed by an 8°C/min temperature increase to 300°C after which it was held at 300°C for 6.5 min. The temperatures of the injector and the flame ionization detectors were 300 and 315°C respectively. The flow rates of

hydrogen, air and nitrogen in the flame ionization detector were 50, 200 and 30 mL/min respectively.

2.2.2 Gas Chromatography/Mass Spectrometry (GC/MS)

Analyses were performed using a Hewlett Packard 5987 GC/MS system, equipped with a cross-linked methyl silicone capillary column with length, internal diameter and film thickness of 25 m, 0.25 mm and 0.33 μm respectively. A helium linear flow velocity of 25 cm/s and a splitless mode injector with the column directly coupled to the mass spectrometer were employed. The temperature of the column was programmed at 45°C for 5 min followed by an 8°C/min temperature increase to 300°C. The injector and ion source temperatures were 300 and 200°C respectively. The electron energy was 70 eV and calibration of the mass axis along with tuning of the ion source were performed using the manual tune features with perfluorotributylamine. The mass range was 41-505 AMU (atomic mass units) and the scan rate was 1.0 scan/second.

2.3 Reference Compounds

The standards were obtained commercially from Ultra Scientific and RFR Corporation.

3. RESULTS AND DISCUSSION

3.1 Solubility

The solubility of the emulsifier and methoxychlor-enriched emulsifier was very poor in hexane and toluene in contrast to the solubility in methylene chloride. That may be indicative of a degree of polarity in the samples. As discussed subsequently, polar organics were not detected.

3.2 Homogeneity

The homogeneity of the samples was poor. The use of spectroscopic data for the purpose of fingerprinting was therefore limited. The method should be employed with caution. For example, as discussed later, significantly different GC/FID chromatograms were determined for subsamples of the same original sample.

3.3 Evaluation of Chromatographic Data

The total hydrocarbon concentrations for the emulsifier and methoxychlor-enriched emulsifier are estimated to be 10% (w/v), most of which are single-ring aromatic hydrocarbons.

3.3.1 GC/FID

The profiles of GC/FID chromatograms of aliquots of the emulsifier corresponding to subsamples taken without shaking for 48 h and immediately after shaking are different. There are no major components above 8.28 min (which corresponds to the C_7-C_{18} hydrocarbon range) for the emulsifier immediately after shaking, in

contrast to peaks occurring at retention times 8.98, 9.53, 10.71, 12.60, 14.58, and 14.85 min (corresponding to the C_{7-12} hydrocarbon range). The profiles at retention times less than 8 min are, however, similar, suggesting that the difference in the chromatograms is not simply a function of volatilization. Changes due to volatilization are likely to be more pronounced for the lower molecular weight hydrocarbons eluting before 8 min (corresponding to the C_4-C_6 hydrocarbon range).

The different profiles are due, in part, to subsampling effects, details of which require further study. The GC/FID chromatogram of the methoxychlor-enriched emulsifier recorded immediately after preparation of the extract closely matches the chromatogram of the emulsifier extract immediately after shaking. Both chromatograms contain prominent low molecular weight hydrocarbons which approximate the C_{5-6} hydrocarbon range. The relative abundance of these components are, however, different and due, in part, to subsampling effects. Methoxychlor was not detected in the fortified emulsifier using GC/FID analysis. Preliminary investigations using gas chromatography/electron capture detection (GC/ECD) gave a complex chromatogram.

3.3.2 GC/MS

Target polynuclear aromatic hydrocarbons listed in Table 1 were not detected in the samples. The samples contained cyclohexane; toluene; o-, m- and p-xylenes; trimethylbenzene, C_9H_{12} alkyl

Table 1. Target polynuclear hydrocarbons.

Naphthalene
Acenaphthylene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(a)pyrene
Dibenzo(a,h)anthracene
Benzo(g,h,i)perylene

benzenes and propyl benzene. Under the conditions employed, polar organics, inorganic components or thermally labile compounds may not be detected by the method.

3.4 Future Work and Conclusion

Fingerprinting of the hydrocarbon content of environmental samples is a challenging problem requiring careful attention to many variables. Special attention must be given to (a) sampling effects in the field and subsampling in the laboratory, (b) reproducibility in the homogeneity of the samples and (c) the time dependence, weathering and aging of the samples. Application of the fingerprinting studies to emulsifier water mixtures, or emulsifier sediment mixtures will introduce the question of the extent of partitioning of the components into other matrices. These are major factors requiring further study.

The samples studied in this work appear to contain a preponderance of non-polynuclear aromatic hydrocarbons and polar or thermally labile components. Since the latter are not detected by the method, it is recommended that high performance liquid chromatographic characterization be included in future work.

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PART IV

ACUTE AND CHRONIC TOXICITY OF METHOXYCHLOR TO FISHES

1. INTRODUCTION

Methoxychlor is a broad-spectrum organochlorine pesticide used, in Alberta, to control emergence of biting flies in the Athabasca River (see Part I). The environmental chemistry and toxicology of methoxychlor have been reviewed by Merna and Eisele (1973), Gardner and Bailey (1975), the National Research Council of Canada (1983), and Murty (1986a, b).

2. MATERIALS AND METHODS

Five separate studies were conducted to assess the toxicity of methoxychlor to fishes. Three of these studies were of an acute nature (96 h) and two were chronic studies (up to 68 d). The acute studies examined the lethality of methoxychlor to a variety of fish species as a function of the duration of exposure, the emulsifier content of the toxicant, and fish age. The chronic studies investigated the effects of methoxychlor on the survival, growth, and development of early life stages of fish. Each of these five studies is described in detail.

2.1 Acute Studies

2.1.1 Experimental Animals

Six species of fish were used in the acute studies: fathead minnow, Pimephales promelas; rainbow trout, Salmo gairdneri; spottail shiner, Notropis hudsonius; walleye, Stizostedion vitreum vitreum; white sucker, Catostomus commersoni; yellow perch, Perca flavescens.

The rainbow trout were a domesticated stock obtained as fertilized eggs from Rainbow Springs Hatchery in Thamesford, Ontario. The walleye were a stock hatched at the Sam Livingston Hatchery in Calgary, Alberta and then transplanted to Plamondon Lake, Alberta, where our experimental fish were captured. All other test animals were stocks captured in Alberta lakes: fathead minnows from Lac Santé; spottail shiners and yellow perch from Marie Lake; white suckers from Gleniffer Lake. Test animals were classified using the keys of Scott and Crossman (1979).

At the Alberta Environmental Centre, the fish were held in a 90% water-reuse system (water source, dechlorinated municipal tapwater) and under a 12 h light/12 h darkness photoperiod. Daily water temperature throughout the holding period averaged (± S.D.) 11.6 ± 0.7°C, pH averaged 7.48 ± 0.05, dissolved oxygen concentration averaged 9.4 ± 0.2 mg/L, and conductivity averaged 238 ± 11 µS/cm. The total chlorine concentration of laboratory holding water was undetectable (<0.01 mg/L). Other physico-chemical characteristics of the laboratory holding water are summarized in Table 1. The fish were fed frozen brine shrimp, live food (small rainbow trout and spottail shiners), and/or commercial dry feeds, as outlined in Table 2. Experimental animals were not fed for at least 24 h prior to, or during the tests, unless indicated otherwise.

Table 1. Physico-chemical characteristics of water used to hold and test fish for acute and chronic studies. The data were obtained by analysis of two grab samples, one collected shortly before studies commenced and one collected shortly after studies were completed.

Characteristic	Concentration (mg/L) 85.06.05 sample	86.04.01 sample
Alkalinity[a]	78	67
Aluminum[b]	0.103	< 0.020
Ammonia[c]	0.014	NA
Arsenic	< 0.0002	< 0.0002
Barium	NA	0.009
Beryllium	< 0.001	< 0.001
Bicarbonate	94	81
BOD	NA	< 1.0
Cadmium	< 0.001	< 0.001
Calcium	12	43
Chloride	5	6
Chromium	< 0.001	< 0.001
Cobalt	< 0.001	< 0.001
COD	NA	8.8
Copper	0.002	0.010
Fluoride	1.02	0.92
Hardness[a]	71	157
Iron[b]	< 0.02	< 0.02
Lead[b]	< 0.003	< 0.003
Magnesium	10	12
Manganese	< 0.008	< 0.008
Molybdenum	< 0.001	< 0.001
Nickel	< 0.001	< 0.001
$NO_2^c + NO_3^c$	1.50	3.08
Nitrite[c]	< 0.05	< 0.05
Phenols	NA	< 0.002
Phosphorus	< 0.006	NA
Potassium	< 0.2	1.3
Selenium	< 0.0002	< 0.0002
Silica	2.6	3.3
Sodium	36	8
Sulphate	70	75
TDS	186	199
Vanadium[b]	< 0.002	0.004
Zinc	0.023	0.014

[a]Expressed as $CaCO_3$.
[b]Extractable. All other characteristics expressed as total.
[c]Expressed as N.
NA = Not Analysed.
BOD = Biological Oxygen Demand.
COD = Chemical Oxygen Demand.
TDS = Total Dissolved Solids.

Table 2. Food types of fish used in acute studies. Food included frozen brine shrimp (FBS) (Nutrafin, Rolf C. Hagen, Incorporated, Edmonton, Alberta), regular Silver Cup (RSC) (Murray Elevator, Murray, Utah), medicated Silver Cup containing 2% oxytetracycline (MSC) (Murray Elevator, Murray, Utah), medicated BioDiet containing 4% oxytetracycline (MB) (Bioproducts Incorporated, Warrenton, Oregon), and live food (LF) (small rainbow trout and spottail shiners).

Fish species	Food types
Fathead minnow	FBS, MSC, MB
Rainbow trout	RSC
Spottail shiner	FBS, MSC
Walleye	FBS, LF
White sucker	MSC, MB
Yellow perch	FBS, MB

Ten individuals of each species were submitted for histological examination prior to commencement of acute studies. That examination included light-microscopic evaluation of the skin, skeletal muscle, brain, eye, gills, heart, alimentary tract, liver, and kidney, among others. No significant lesions were found in examined tissues of the rainbow trout. As one might expect, that was not the case for feral stocks. The fathead minnows showed moderate hypercellularity and fusion of gill lamellae. Flukes were also present in the brain and peritoneal cavity. Yellow perch showed moderate hypercellularity and fusion of gill lamellae, as well as flukes in the brain. Granulomatous myositis and peritonitis were noted, and marked hepatic

fatty changes were seen. White suckers showed mild to moderate hypercellularity and shortening of gill lamellae. Flukes were present in the alimentary tract, without pathological alterations. Multiple visceral granulomata were found, involving the liver and pancreas. Hepatic fatty changes were noted. Walleye showed necrosis of the haemopoietic tissues of the anterior kidney. Necrotic erythrocytes were seen, especially in blood vessels of the brain. Clusters of protozoan parasites were found in the brain, and flukes were present in the brain and eye. The spottail shiners were heavily parasitized with Myxosporidia cysts, digenetic trematode larvae, and flukes present in the brain. Flukes were also found in the eye, peritoneal cavity and bile duct. Granulomatous hepatitis, encephalitis, myositis, peritonitis, arteritis and osteomyelitis were noted, together with mild keratitis and anterior uveitis. Haemorrhage and necrosis of the liver were found, suggestive of parasitic larval migration. Hepatic fatty changes were evident.

Mortality during the laboratory holding period was judged to be acceptable for each species (<10%), based on recommendations of the American Public Health Association et al. (1980).

2.1.2 Time-Toxicity Studies

Effects of exposure duration on the lethality of methoxychlor were assessed in all six fish species. The tests were conducted in 20-L polyethylene-lined tanks under either static-flow or partial-replacement conditions. During partial replacement tests, 75% of each

test solution was replaced daily. Tanks were mildly aerated throughout both static-flow and partial replacement tests. Each test included a control and five to seven toxicant concentrations. Industrial-grade methoxychlor (Sanex-methoxychlor 25E, registration number 15092, Sanex Incorporated, Mississauga, Ontario), a mixture of 25% methoxychlor in a proprietary emulsifier, was used as the toxicant. Laboratory holding water was used as the diluent.

Fish for a given test were distributed among the tanks (5-11 fish per tank) using a random process and were allowed 48 h to acclimate to the test environment without toxicant. Dead fish were counted and removed from each tank at least once daily. LC_{50} values and their 95% confidence limits (95% CL) were calculated by the trimmed Spearman-Karber method (Hamilton et al., 1977).

Five different exposure or "pulse" regimes were tested. The first was conducted under static-flow conditions and the toxicant was added as a single spike at time zero. All other regimes involved 75% daily replacement of each test solution. These latter regimes consisted of: 2-h exposure to toxicant followed by transfer of the fish to fresh laboratory water for 94 h; 6-h toxicant exposure followed by 90 h in fresh laboratory water; 24-h toxicant exposure followed by 72 h in fresh laboratory water; 96-h toxicant exposure.

Analysis of the test solutions for methoxychlor was performed once or twice during each test. Water samples for methoxychlor analysis were collected at the beginning and end of static-flow tests, and at the approximate midpoint of each pulse exposure in replacement

tests (e.g. after 1 h into 2-h exposures). Methoxychlor was measured as described in Part II. Other physico-chemical characteristics of the test solutions (temperature, pH, dissolved oxygen concentration, conductivity) were measured two or three times in each tank during each test using a Hydrolab Environmental Data System (Hydrolab Corporation, Austin, Texas).

2.1.3 Emulsifier Studies

Lethality of the emulsifier present in Sanex-methoxychlor 25E was assessed using rainbow trout. The tests were conducted in 20-L polyethylene-lined tanks under partial replacement conditions (75% replacement of each test solution daily). Test tanks were mildly aerated. Each test included a control and five toxicant concentrations. Mixtures of reagent-grade methoxychlor (98% pure, product number M7757, Sigma Chemical Company, St. Louis, Missouri) dissolved in 95% ethanol and the Sanex proprietary emulsifier were used as toxicants. The emulsifier was provided by Sanex Incorporated (Mississauga, Ontario). The mixtures tested were, as a weight:weight ratio of methoxychlor to emulsifier, 0:1 (0% methoxychlor), 1:6 (14.3% methoxychlor), 1:3 (25% methoxychlor), 2:3 (40% methoxychlor), and 1:0 (100% methoxychlor). In addition, because the methoxychlor was prepared as a concentrated solution (500 mg/L) in 95% ethanol, the ethanol content of each test solution including the control was adjusted to 3.84 mL, equivalent to a final concentration in each tank of 157 mg/L ethanol. That concentration is approximately

1% of the 96-h LC_{50} value for ethanol to rainbow trout (Johnson and Finley, 1980). Laboratory holding water was used as the diluent.

Fish for a given test were distributed randomly among the tanks (10 fish per tank) as described earlier for the time-toxicity studies. Mortality checks and physico-chemical characterization of the test solutions, including methoxychlor determinations, were also performed as described. Quantification of the emulsifier concentration in test solutions was not possible in our hands. Our attempts to characterize the emulsifier chemically are summarized in Part III.

2.1.4 Fish Age-Toxicity Studies

The acute lethality of methoxychlor was assessed as a function of the age of 1- to 7-week-old (posthatch) fathead minnows. The minnows were obtained from the Alberta Environmental Centre's in-house breeding colony. That colony of fathead minnows was set up and maintained following methods outlined by the American Public Health Association et al. (1980) and using fish originally captured in Lac Santé, Alberta. Breeding adults, their eggs, and resultant larvae and juveniles were held in laboratory water (Section 2.1.1 and Table 1) at 23.8 ± 1.8°C.

The 96-h lethality tests were conducted in 12-L glass containers under flow-through conditions. Each container had a 2-L glass header tank (Figure 1). Test solution flowed under gravity from the header

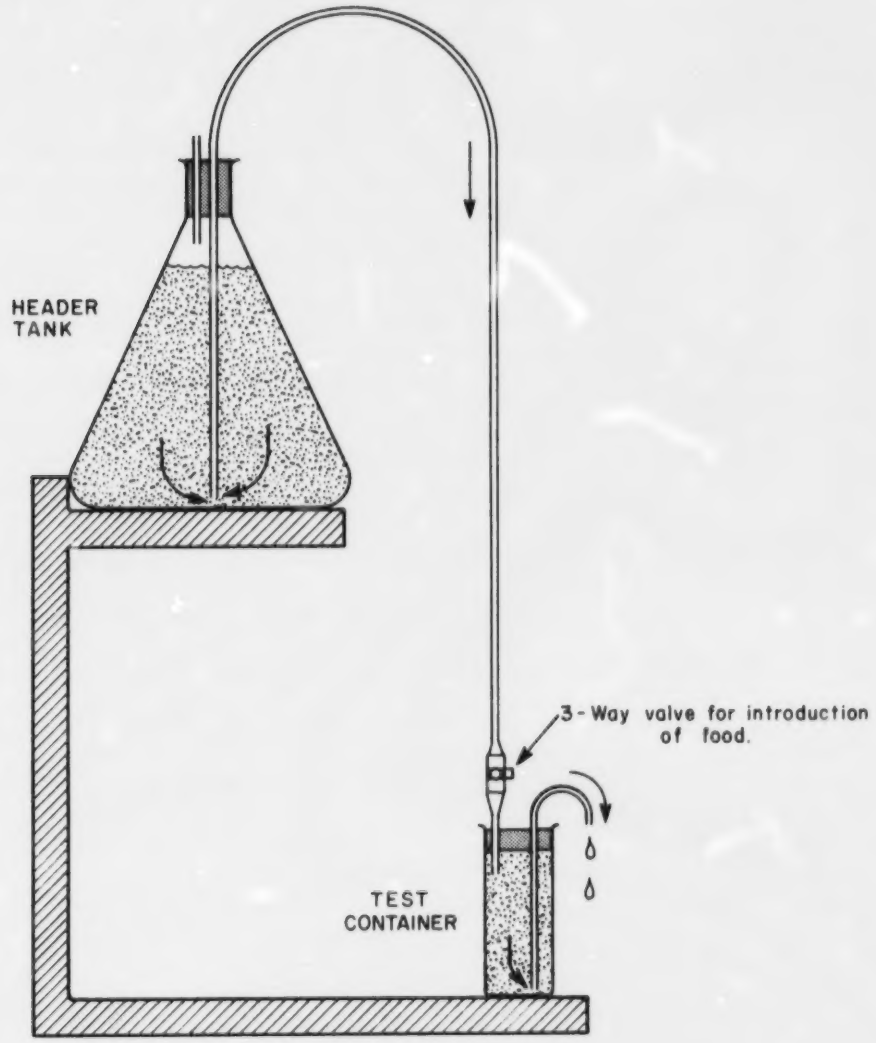

Figure 1. Schematic diagram of the test apparatus used in fish age-toxicity studies with fathead minnows. Arrows indicate direction of toxicant flow.

tank to the test container at a rate of 79 to 102 mL/min, providing 99% molecular replacement of the solution in each test container every 35-50 min [extrapolation of Sprague's (1973) Figure 1]. Freshly prepared test solution was added to each header tank 2 or 3 times daily. The age-toxicity tests were conducted at room temperature (approximately 20°C). Each test included a control and five toxicant concentrations. Industrial-grade methoxychlor (Sanex-methoxychlor 25E) was used as the toxicant. Laboratory water was used as the diluent.

Fish for a given test were distributed randomly among the containers (8-10 fish per container) and were allowed a minimum of 24 h to acclimate to the test environment without toxicant. Test minnows were fed once daily with live (less than 24 h old) brine shrimp nauplii. Dead fish were counted and removed from each container at least once daily.

Analytical constraints prevented the determination of methoxychlor in the test solutions of age-toxicity studies. For this reason, every effort was made to minimize loss of toxicant to the atmosphere, by minimizing air contact, and loss to the walls of the exposure apparatus, by using glass wherever possible. In interpreting the results, however, the reader should note that nominal toxicant concentrations were used to calculate LC_{50} values in age-toxicity studies.

2.2. Chronic Studies

Whereas the acute studies used death as an endpoint , the chronic studies incorporated a number of behavioural and physiological end-points. These included hatching, growth and development. In this way, the chronic studies provided information on the viability or survival potential of an exposed fish population. Acute lethality tests, on the other hand, say nothing about whether or not exposure adversely affects those functions upon which survival of a population is ultimately dependent (e.g., maturation, growth, feeding, reproduction, predator avoidance).

2.2.1 Hatching Studies

Studies were designed to examine the effects of a 2-h pulse of methoxychlor on the hatching behaviour and success of rainbow trout. Rainbow trout were obtained as fertilized eggs from Rainbow Springs Hatchery in Thamesford, Ontario. At the Alberta Environmental Centre, the eggs were held in commercial incubation trays (Heath Techna-Plastics Incorporated, Auburn, Washington). Each tray was subdivided into 12 equivolume compartments by plexiglass panels. The eggs were enumerated manually and randomly distributed to a final density of 100 eggs per compartment. The incubation trays were kept in darkness and were provided with flowing laboratory water. The physico-chemical characteristics of this water are summarized in Section 2.1.1 and Table 1.

Approximately one week before hatching was anticipated, some of the eggs were exposed to methoxychlor. Eggs in individual compartments were gently transferred to nylon-mesh bags. The bags were then suspended in polyethylene-lined tanks containing 20 L of test solution. Test solutions were mildly aerated. The bags were manually moved through the water column at 15 min intervals to aid water exchange through the mesh walls. After 2-h exposure, the bags were rinsed in laboratory holding water, then the eggs removed from the bags and returned to the appropriate compartments of the incubation trays. The study included a control and 4 toxicant concentrations, nominally 0, 150, 300, 600 and 1200 µg/L as methoxychlor. Five bags, equivalent to 5 groups of 100 eggs each, were suspended in each test solution. Sanex-methoxychlor 25E was used as the toxicant. Laboratory holding water was used as the diluent.

Following methoxychlor exposure, records were made at least once daily of the number of dead eggs, the total number of hatchlings (alevins) and the number of dead alevins. Alevin survival was monitored for 18 days posthatch. Dead eggs and alevins were immediately removed.

Approximately three days after hatching occurred, some of the resultant alevins were exposed to methoxychlor. Both previously exposed and naive (not previously exposed) alevins were used. The exposures were performed as described for eggs. The only difference was that the animals exposed to each test solution included 5 groups of 100 naive alevins each and 2 groups of 10 previously exposed

alevins each. One of these latter groups was fish that had been exposed as eggs to 0 µg/L (control), while the other group was fish that had been exposed as eggs to 300 µg/L methoxychlor (nominal concentration). Alevin survival was monitored for 15 d after exposure.

Water samples for methoxychlor analysis were collected immediately after each 2-h exposure of eggs or alevins. Methoxychlor was measured as described in Part II.

2.2.2 Studies of Embryonic and Alevin Development

Studies were undertaken to investigate the effects of methoxychlor on the early growth and development of rainbow trout. The trout were obtained as freshly fertilized eggs from the Raven Brood Station, near Caroline, Alberta. The eggs were less than 24 h old (postfertilization) when received at the Alberta Environmental Centre. Upon receipt, the eggs were counted volumetrically and distributed randomly to three experimental tanks. These tanks were the same ones used in microcosm studies (Part V). The plexiglass tanks contained 680 L of test solution under thermostat-controlled, recirculated-flow conditions. For our purposes, the bottom of each tank was covered with a layer of prewashed, 20-mm crushed gravel. The eggs themselves were placed in a screen compartment (91x51x51 cm) at one end of the tank in such a way that the entire flow of recirculated water (5 L/min) passed over the eggs. Approximately 1,000 eggs were placed in the compartment of each tank. Egg compartments were kept in darkness. Approximately 12 h after receipt of the eggs (egg age,

1.5 d postfertilization), the tanks were spiked with Sanex-methoxychlor 25E to nominal concentrations of 0, 3 and 30 µg/L as methoxychlor. Laboratory water was used as the diluent (Section 2.1.1 and Table 1).

Water samples for methoxychlor analysis were collected approximately 2 h after the spike. Egg/alevin samples for growth and development analyses were collected at experimental time zero (about 2 h after the methoxychlor spike) and routinely thereafter. Dead eggs and alevins were counted and removed at least once daily. Physico-chemical characteristics of each test solution (temperature, pH, dissolved oxygen concentration, conductivity) were measured routinely using a Hydrolab Environmental Data System (Hydrolab Corporation, Austin, Texas).

When the embyros developed eye pigment (Vernier's [1969] morphological stages 21-22) all the eggs in a tank were removed, mechanically shocked, manually counted and then returned to the tank. Mechanical shocking is a standard hatchery procedure that facilitates the separation of viable from nonviable eggs on the basis of a simple colour difference. At this time, some of the viable eggs (n = 50) were returned to a floating mesh basket (11x11x13 cm) in the tank. Eggs in the floating basket were employed to monitor the timing of hatch (time of 50% hatch and duration of 95% hatch).

Eggs and alevins for growth and developmental analyses were euthanized with tricaine methane sulfonate (MS 222) and preserved in 10% buffered formalin for at least 15 d. This period of time is more

than adequate to allow for stabilization of the morphometric changes associated with fish preservation (Heming and Preston, 1981). Preserved fish were blotted to remove excess surface water and then weighed to the nearest 0.1 mg. Embryo and alevin fork length was measured to the nearest 0.5 mm. Where applicable, the egg capsule, yolk and tissues of each individual were separated by dissection, weighed, then dried to constant weight at 60°C and re-weighed. Fish were staged using the morphological stages of Vernier (1969). For each sampled individual, we recorded clock age (days post-fertilization), physiological age (thermal units postfertilization), morphological stage (after Vernier, 1969), developmental index [k_D = 10 (total wet weight)$^{1/3}$/length, Bams (1970)], fork length, total wet weight, dry weight and percent water content, tissue wet weight, dry weight and percent water content, yolk wet weight, dry weight and percent water content, egg capsule wet and dry weight, and perivitelline fluid weight. We then used the means of all observations at each sampling time to calculate the rate of morphological development, tissue growth rate, yolk absorption rate, and yolk-to-tissue conversion efficiency.

3. RESULTS AND DISCUSSION

3.1 Time-Toxicity Studies

Two static-flow tests were run, one each with walleye and yellow perch. In both cases, the exposure concentration decreased significantly over the course of the test (Table 3), presumably due to

Table 3. Change in the methoxychlor concentration (µg/L) of test solutions in static-flow tests. Fish mortality during 96-h static-flow tests with walleye and yellow perch.[a]

Nominal exposure concentration	Measured methoxychlor concentration		Percent loss of methoxychlor	Percent 96-h mortality[b]
	At time zero	After 96 h		
		WALLEYE TEST[c]		
0	0.18[f]	0.10[f]	--	0
5	3.7	0.25	93	14
10	6.7	0.42	94	0
20	15	1.9	87	43
40	37	1.2	97	71
80	75	10	87	100
		YELLOW PERCH TEST[d]		
0	0.11[f]	0.12[f]	--	0
5	6.0	0.16	97	0
10	13	0.32	98	0
20	15	0.50	97	0
40	28	1.1	96	0
80	61	1.8	97	0
80[e]	99	8.9	91	--

[a]Mean with range, water temperature 11.1°C (10.3-11.7), pH 7.8 (7.6-7.9), dissolved oxygen concentration 7.8 mg/L (6.9-9.3), conductivity 362 µS/cm (358-368).
[b]Corrected for control mortality in walleye test.
[c]n = 10 fish per tank. Mean with range, fish weight 3.1 g (2.0-5.3), fork length 7.5 cm (6.1-9.6).
[d]n = 10 fish per tank. Mean with range, fish weight 2.1 g (0.9-5.1), fork length 6.0 cm (4.4-8.1).
[e]No fish present in this tank.
[f]Values considered analytical or experimental artifacts; method detection limit is 0.03 µg/L.

sorption of methoxychlor to the walls of the experimental apparatus and loss to the atmosphere via volatilization. Partitioning of methoxychlor to the experimental fish also reduced the exposure concentration, as evident from the greater loss of methoxychlor in the presence of yellow perch (approximately 97%) than in their absence (91%) (Table 3, yellow perch test).

The loss of methoxychlor throughout static-flow tests made it impossible to calculate LC_{50} values by conventional methods. Still, mortality during these studies can be expected to be a function of toxicant exposure (i.e., exposure concentration and duration). Assuming that methoxychlor exposure was a function of the area under the concentration-time curve and, for simplicity, that the decrease in methoxychlor concentration was linear with time, one can calculate the concentration-time value (µg•h/L) associated with 50% mortality. We completed these calculations and found 50% mortality of walleye to occur at a concentration-time value of 900 µg•h/L (95% confidence limits, 520-1,500). Dividing this value by 96 h, one obtains a 96-h LC_{50} of 9.4 µg/L (95% confidence limits, 5.4-16) for walleye. None of the yellow perch died during their static-flow test and, consequently, we calculated their 96-h LC_{50} to be greater than 31 µg/L. Because of the theoretical uncertainties of such calculations, these LC_{50} values should be regarded only as estimates of the actual 96-h LC_{50} values.

In partial-replacement tests, the concentration of methoxychlor fluctuated on a daily basis as the continual loss of methoxychlor to

the atmosphere, apparatus walls and experimental fish was partially offset by daily replacement of 75% of each test solution. The magnitude of these temporal fluctuations was not measured. Instead, determination of the methoxychlor concentration at the approximate midpoint of each pulse, that is, after 1 h into 2-h exposures, 3 h into 6-h exposures, 12 h into 24-h exposures and 50 h into 96-h exposures, was assumed to provide a concentration value representative of the entire exposure.

In 96-h exposures, the relationship between the measured concentration (y, µg/L) and the nominal (time zero) concentration (x, µg/L) was best described as:

[1] $y = 0.46x - 1.19$

($r^2 = 0.974$). In other words, the measured concentration at the midpoint between daily solution replacements was less than half the nominal (time zero) concentrations. In 2-, 6- and 24-h exposures, the measured concentrations after 1, 3 and 12 h into the respective pulses were 0.74, 0.71 and 0.25 of the nominal (time zero) concentrations (r^2= 0.913, 0.996 and 0.968 respectively for the linear regression of the measured values on the nominal values). The information was used to model the decrease in methoxychlor concentration of the test solutions (y, fraction of methoxychlor concentration remaining) with time (x, h). The resulting relationship,

[2] $\ln y = -0.103 - 0.082x$

($r^2 = -0.905$), predicts that after 24 h only 0.13 of the initial methoxychlor concentration remained in any given test solution. This

is in good agreement with the temporal changes in methoxychlor concentration found in Part V for microcosms.

Results of the partial-replacement tests are summarized in Figure 2. For each species, the 96-h pulsed LC_{50} [1] increased significantly as the pulse duration decreased. In other words, fish tolerated exposure to a higher concentration of methoxychlor for a shorter period of time than could be tolerated for a longer period of time. The relationship between the 96 h pulsed LC_{50} (y, µg/L) and the pulse duration (x, h) for rainbow trout was best described as:

[3] $\ln y = 7.195 - 1.086 \ln x$

($r = -0.999$). We used this model to extrapolate the 96 h pulsed 15-min LC_{50} for rainbow trout and obtained a value of 6,000 µg/L, equivalent to 20 times the nominal concentration of 300 µg/L employed in treating the Athabasca River. Using similar models for the other test species, we extrapolated 96-h pulsed 15-min LC_{50} values (in µg/L) of 280 for walleye, 55,000 for spottail shiner, 90,000 for fathead minnow, and 161,000 for white sucker. Hence, it would appear that, of all the fishes studied, walleye were the most sensitive to short pulses of methoxychlor. Moreover, their predicted 96-h pulsed 15-min LC_{50} is close to, if not lower than, the nominal treatment concentration used in the Athabasca River. The 96-h pulsed 15-min LC_{50} values of the other test species were approximately

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[1] The format used when referring to pulsed bioassay data is: "duration of study" pulsed- "duration of pulse" - LC_{50}. For example, the 96-h pulsed 2-h LC_{50} is the median lethal concentration over a 96-h total study period for fish exposed to a 2-h pulse (i.e., 2-h toxicant exposure followed by 94 h in fresh laboratory water).

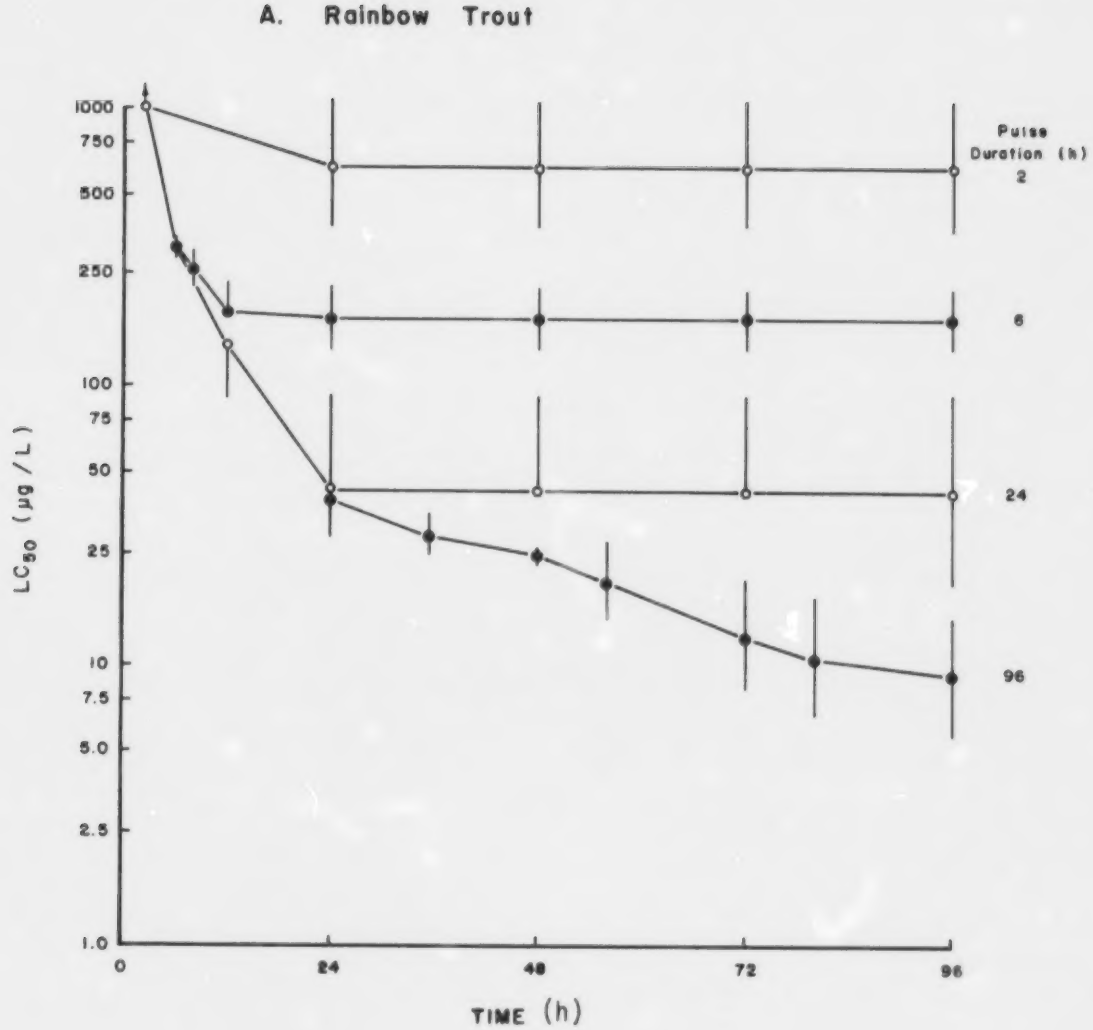

Figure 2. Time-toxicity relationship for methoxychlor to fishes, as a function of pulse (exposure) duration. Data points are LC_{50} values with the 95% confidence limits. Physico-chemical characteristics of the tests were (means with range): water temperature 10.6°C (9.9-11.8); pH 7.8 (7.3-8.1); dissolved oxygen concentration 10.6 mg/L (8.2-12.1); conductivity 343 µsiemens/cm (322-363). PANEL A -- rainbow trout. n = 10 fish per tank. Mean with range, fish weight 1.2 g (0.3-2.9), fork length 4.9 cm (2.8-6.5).

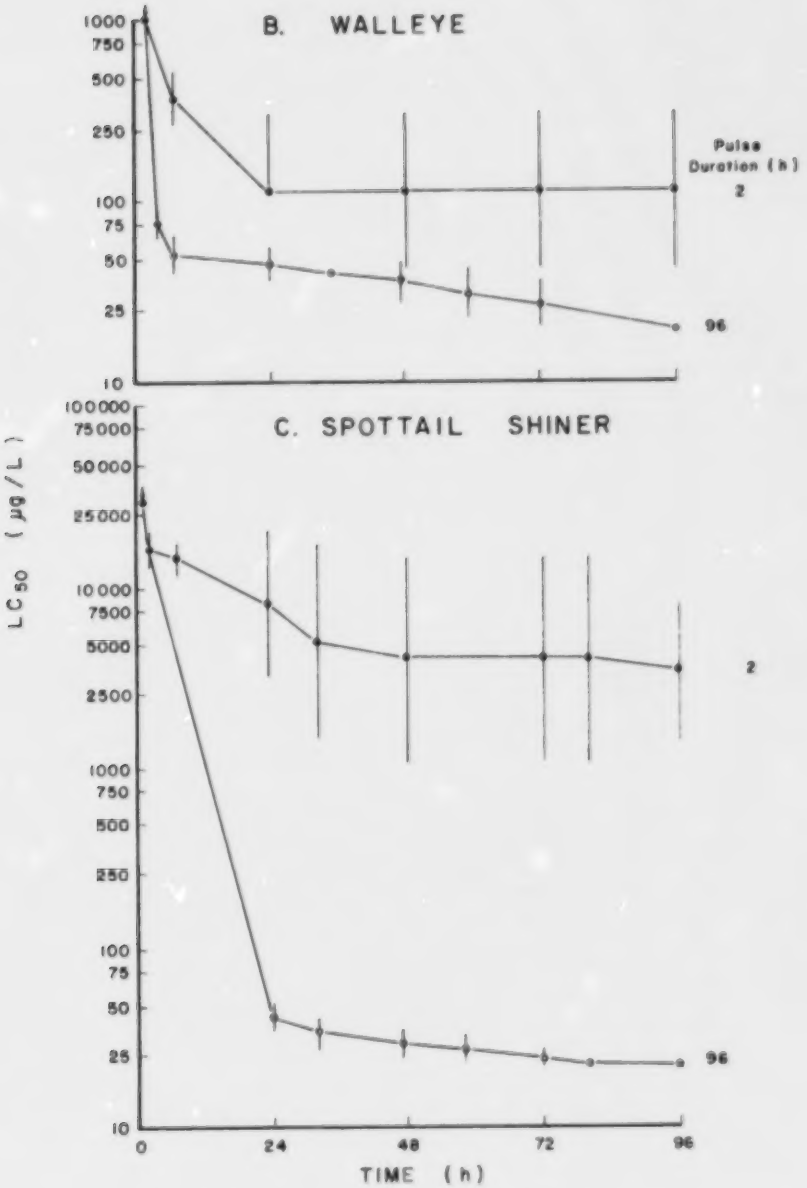

Figure 2. continued

PANEL B -- walleye. n = 5 fish per tank. Mean with range, fish weight 5.2 g (2.7-8.4), fork length 8.5 cm (7.3-10.1). PANEL C -- spottail shiner. n = 9-10 fish per tank. Mean with range, fish weight 1.5 g (0.4-3.0), fork length 5.3 cm (3.6-6.4).

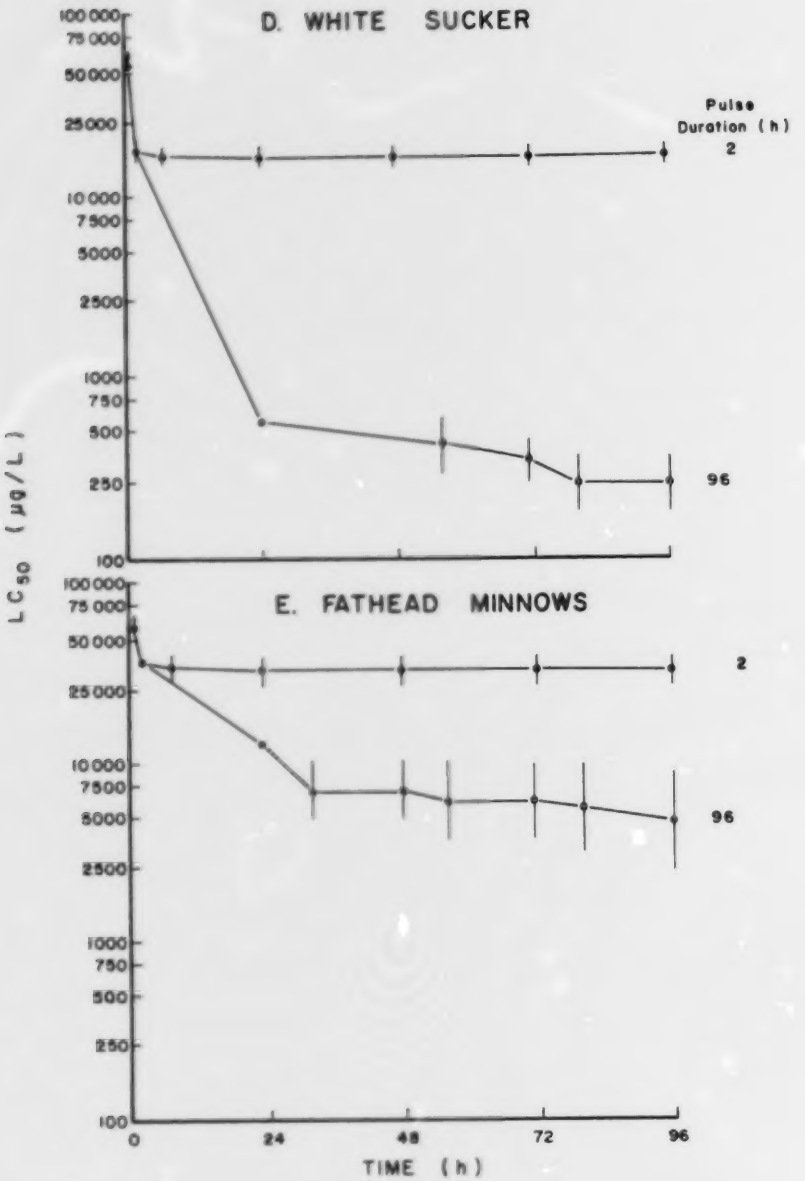

Figure 2. continued

PANEL D -- white sucker. n = 8-11 fish per tank. Mean with range, fish weight 3.3 g (0.7-9.3), fork length 6.7 cm (4.1-9.7). PANEL E -- fathead minnow. n = 10 fish per tank. Mean with range, fish weight 2.3 g (0.7-3.9), fork length 6.0 cm (4.3-7.0).

200-500 times greater than the treatment concentration used in the Athabasca River. Comparison of time-toxicity relationships among the various test species must be tempered, however, by the information on interspecific differences discussed later.

With the exception of the fathead minnow data, 96-h LC_{50} values (equals 96-h pulsed 96-h LC_{50}) determined in the present study agree reasonably well with literature values (Table 4). Review of the literature indicates that 96-h LC_{50} values for methoxychlor have been determined in at least 24 species of fish encompassing 6 orders. In overview, there is little evidence of interspecific differences, especially in light of the apparent intraspecific and/or inter-laboratory differences. The reported LC_{50} values for individual species vary by as much as an order of magnitude. To examine this point, we determined the intraspecific difference in methoxychlor toxicity to rainbow trout. In studies conducted under identical experimental conditions, we found the 96-h LC_{50} ($\pm$ 95% CL) of 6.7-g rainbow trout (range 2.9-15.1) from Sun Valley Hatchery, Mission, British Columbia, to be 31 µg/L (25-40), whereas the value for 1.2-g rainbow trout (range 0.3-2.9) from Rainbow Springs Hatchery, Thamesford, Ontario, was significantly lower, 9.4 µg/L (5.7-15). Given the differences in fish size, age and health among the present test species, and the uncertainty in present exposure concentrations, conclusions derived from interspecific comparison of the present results should be regarded as tentative at this time. Thus, the

Table 4. Summary of acute toxicity thresholds for methoxychlor to juvenile and adult fishes.

Order	Fish species	96-h LC_{50} (µg/L)	Source of information
Atheriniformes	Atlantic silverside	33	1
	Flagfish	290	3
	Guppy	120	2
	Mummichog	46	1
	Striped killifish	30	1
Cypriniformes	Fathead minnow	8.5	7
	Fathead minnow	39	4
	Fathead minnow	64	2
	Fathead minnow	4,700	8
	Goldfish	42	5
	Goldfish	42	4
	Goldfish	56	2
	Spottail shiner	24	8
	White sucker	260	8
Perciformes	Bluegill	29	5
	Bluegill	32	4
	Bluegill	40-62	2
	Bluehead	13	1
	Largemouth bass	16	4
	Striped bass	3.3	6
	Striped mullet	63	1
	Yellow perch	18-30	4
	Yellow perch	20	7
	Walleye	20	8
Salmoniformes	Atlantic salmon	1.7-16	4
	Brook trout	12-19	4
	Cutthroat trout	15	4
	Lake trout	17	4
	Northern pike	12	4
	Rainbow trout	9.4-31	8
	Rainbow trout	62	4
Siluriformes	Channel catfish	52	4
Tetraodontiformes	Northern puffer	150	1

SOURCES:
1. Eisler, 1970.
2. Henderson et al., 1959.
3. Holdway and Dixon, 1985.
4. Johnson and Finley, 1980.
5. Kennedy et al., 1970.
6. Korn and Ernest, 1974.
7. Merna et al., 1972.
8. The present study.

relative sensitivity of walleye to short pulses of methoxychlor must be treated with a certain degree of skepticism.

The 96-h LC_{50} obtained for fathead minnows under the present experimental conditions (4,700 µg/L, 95% CL 2,400-9,000) is substantially greater than the literature values for that species or other fish species. Statistically, the probability of obtaining such a high value given the data set in Table 4 is less than 0.1% and, consequently, the value can be considered an outlier. There are several possible explanations of how such an anomalous result could have arisen. Firstly, the value could be an experimental artifact arising from the peculiar behaviour of exposed minnows. In the present study, fathead minnows exposed to methoxychlor bloated with gas and lost their ability to maintain station in the water column; the fish floated upside down at the water surface. Gross histological examination indicated the bloating was due to distension of the swimbladder and gastrointestinal tract. Bloating was primarily, but not solely, due to the swallowing of atmospheric air; some minnows bloated even when denied access to the water surface by a screen. This suggests that at least some of the bloating gas originated from over-inflation of the swimbladder and the subsequent release of swimbladder gas into the gastrointestinal tract via the pneumatic duct. Fathead minnows were the only test species to show such bloating behaviour. Perhaps, by floating upside down at the surface, fathead minnows were exposed to less methoxychlor than one would predict based on measurement of the bulk concentration and,

consequently, the reported LC_{50} value may overestimate the actual median lethal concentration. Although not lethal in itself, the bloating behaviour of exposed fathead minnows would probably reduce their survival potential in field situations.

Secondly, the seemingly high 96-h LC_{50} for fathead minnows could be an artifact attributable to the large temporal fluctuations in exposure concentration. During 96-h exposures, the methoxychlor concentration varied daily between approximately 100% and 13% of the time zero value. If the test minnows were able to biotransform and excrete methoxychlor at relatively rapid rates, they would be able to substantially reduce their body burden of toxicant on a daily basis as the exposure concentration decreased with time. This would invalidate the direct comparison of LC_{50} values obtained under fluctuating exposure concentrations, as in the present static and partial-replacement studies, and values obtained under stable exposure concentrations, as in flow-through studies. This possible explanation is supported by results of the age-toxicity studies with fathead minnows which were conducted under flow-through conditions (see Section 3.3). These latter studies yielded LC_{50} values more in keeping with the literature values for methoxychlor toxicity to fathead minnows (Table 4).

A third possibility is that the feral stock of fathead minnows used in the present study was resistant to organochlorine pesticides such as methoxychlor. Pesticide resistance in fish can develop within a short period (weeks) of exposure or can be inherited from an

ancestral stock that had been exposed to pesticide residues (for review and references, see Murty, 1986a,b). Moreover, since cross-resistance to structurally similar compounds has been demonstrated in fish, prior exposure to methoxychlor per se is not a requirement for methoxychlor resistance. While an unlikely scenario, methoxychlor resistance cannot be ruled out because the history of the test stock is unknown.

Methoxychlor had delayed toxic effects in each test species; fish continued to die and the LC_{50} value decreased significantly after fish in 2-h pulse studies were transferred to fresh laboratory water (Figure 2). These delayed mortalities continued for approximately 24 h after transfer to fresh laboratory water. This suggests that evaluation of fish mortalities in field situations should continue for at least 24 h after methoxychlor treatment.

Figure 3 summarizes the temporal changes in methoxychlor concentration measured in the Athabasca River during the May, 1986 treatment and in microcosm tests (see Part V). Comparison of the data in Figure 3 with the present time-toxicity data (Figure 2, Part VI) leads one to conclude that methoxychlor concentrations in the Athabasca River probably never reach the threshold for acute fish lethality, except within the immediate downstream mixing zone. A low exposure concentration probably accounts for the absence of reported fish kills following methoxychlor treatment of the Athabasca River. Fish avoidance of methoxychlor pulses is not necessary to account for the absence of reported fish mortalities.

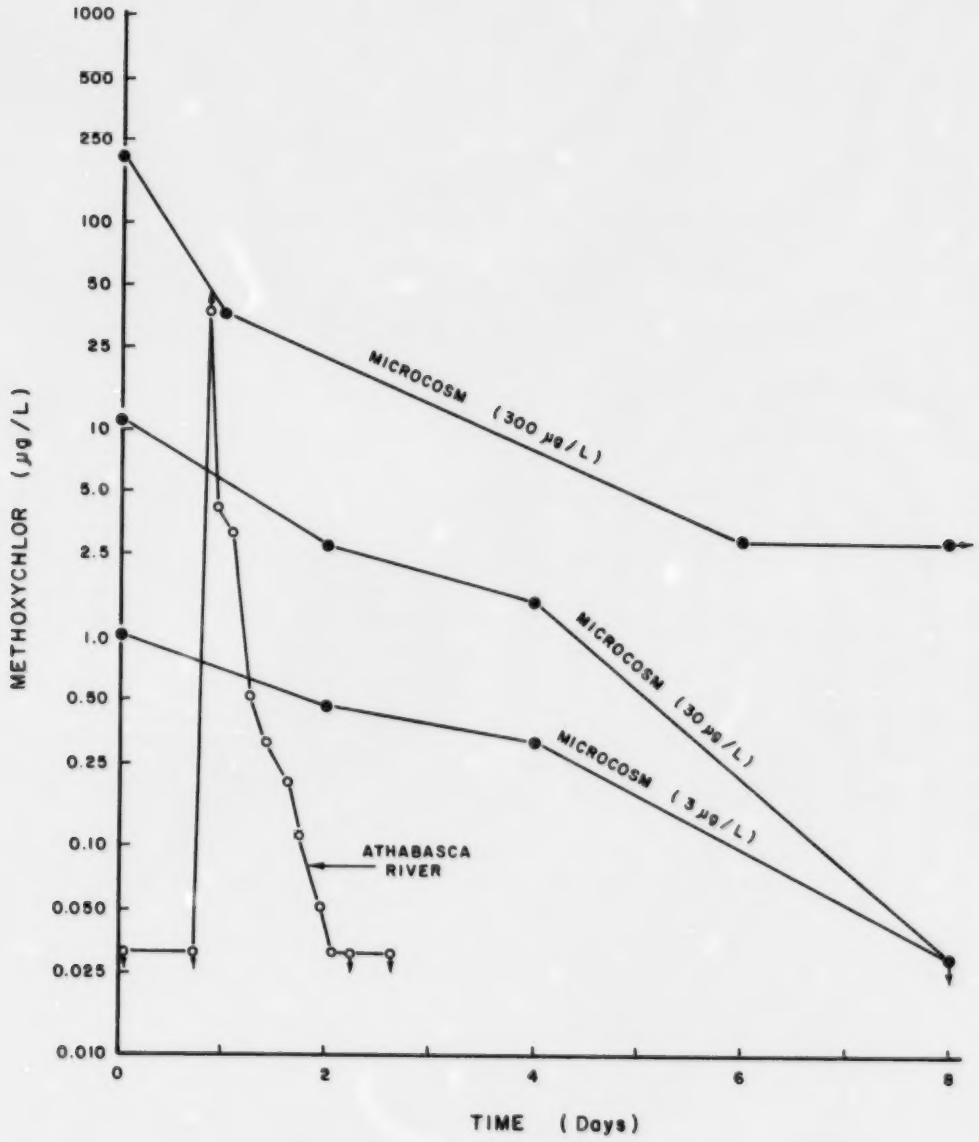

Figure 3. Temporal changes in the methoxychlor concentration of the Athabasca River during the May, 1986 treatment and in microcosm tests. Athabasca River data are from McLeod (1987). The river was treated on May 29 (time zero) at km 80 at a nominal concentration of 297 µg/L and then again on May 30 at km 160 at a nominal concentration of 298 µg/L.

3.2 Emulsifier Studies

Results of the emulsifier studies are summarized in Table 5. We were unable to measure the emulsifier concentration of test solutions and, therefore, calculated the LC_{50} values for methoxychlor/emulsifier mixtures in terms of methoxychlor content. Nominal exposure concentrations were used in calculating the LC_{50} of pure emulsifier. Because of the volatile nature of the emulsifier, these nominal values probably significantly overestimated the actual exposure concentration and, hence, the reported LC_{50} of Sanex proprietary emulsifier probably overestimates the actual median lethal concentration. In other words, the emulsifier is probably more toxic than indicated by the present LC_{50} value (Table 5). It would appear, nonetheless, that pure emulsifier was much less toxic than pure methoxychlor.

In field applications of Sanex-methoxychlor 25E, fish are exposed to both emulsifier and methoxychlor. There is good evidence of interactions between certain carrier solvents and organochlorine pesticides resulting in a more-than-additive toxic effect (Murty, 1986b). The potential for such synergistic effects would not be apparent from studies of pure compounds alone or of a single mixture. For this reason, we determined the interactive toxicity of reagent-grade methoxychlor and the Sanex emulsifier using methoxychlor: emulsifier mixtures of 1:0, 2:3, 1:3, 1:6, and 0:1 (Table 5). If the emulsifier was appreciably toxic itself or synergistically enhanced the toxicity of methoxychlor, then the LC_{50}

Table 5. Acute toxicity of methoxychlor/emulsifier mixtures to rainbow trout.[a]

Ratio methoxychlor:emulsifier	Percent methoxychlor	96-h LC_{50} with 95% CL (µg/L)
1:0	100	6.5 (4.9- 8.7)[b]
2:3	40	9.4 (3.9-22)[b]
1:3	25	11 (NC)[b]
1:6	14.3	12 (NC)[b]
0:1	0	13,000 (11,000-16,000)[c]

[a]n = 10 fish per tank. Mean with range; water temperature 10.5°C (10.1-10.8), pH 7.4 (7.0-7.8), dissolved oxygen concentration 9.4 mg/L (7.1-11.8), conductivity 317 µsiemens/cm (304-337), fish weight 1.7 g (0.5-5.7), fork length 5.4 cm (3.8-7.9).
[b]Expressed as methoxychlor.
[c]Expressed as emulsifier and calculated using nominal exposure concentrations.
NC = not calculable by method used.

of such mixtures (expressed as methoxychlor) would decrease as the relative proportion of emulsifier in the mixture increased; less methoxychlor would be required to produce median mortality in the presence of emulsifier. That was not observed; if anything, the LC_{50} of the mixtures (expressed as methoxychlor) tended to increase as the proportion of emulsifier increased. This supports the conclusion that the emulsifier was much less toxic than methoxychlor and suggests that emulsifier and methoxychlor interacted to produce simple additive or possibly less-than-additive toxic effects.

3.3 Fish Age-Toxicity Studies

The acute lethality of Sanex-methoxychlor 25E to fathead minnows was unaffected by fish age between 1 and 7 weeks posthatch (Table 6). The absence of an age effect probably indicates that widely different life stages were not examined. It is well known that different life stages of fish differ in their sensitivity to organochlorine pesticides (Murty, 1986a,b). Generally, egg (embryonic) stages are the least sensitive, larval stages are the most sensitive, and juvenile and adult sensitivities are intermediate between the two extremes. A review of the literature suggests this trend holds true for methoxychlor. Johnson and Finley (1980) report LC_{50} values for brook trout, Salvelinus fontinalus , of >50,000 µg/L for eyed eggs, decreasing to ~400 µg/L for yolk-sac fry (alevins). Young juvenile striped mullet, Mugil cephalus, are more sensitive to methoxychlor than older juveniles (Lee et al., 1975). Likewise, Holdway and Dixon (1985) found methoxychlor sensitivity to decrease with age between 2 and 8 days posthatch in flagfish, Jordanella floridae, when the fish were fed. Conversely, they found no effect of age when the fish were starved. Similarly, Holdway and Dixon (1984) showed the methoxychlor sensitivity of white suckers to decrease with age between 2 and 26 days posthatch when fed, but to increase with age when starved.

The present LC_{50} values for young fathead minnows were 1.4-2.9 µg/L, in reasonable agreement with literature values for the species (Table 4). The values determined in age-toxicity studies were

Table 6. Acute toxicity of methoxychlor to fathead minnows, as a function of fish age (weeks posthatch).

Fish age (weeks)	Average fish weight (mg)	Average fish length (mm)	96-h LC_{50} with 95% CL (µg/L)[a]
1-2	1.4	6.1	1.8 (0.85-3.7)
3-4	2.2	6.2	1.4 (0.75-2.6)
6-7	33.1	16.7	2.9 (1.1 -7.7)

[a]n = 8-10 fish per container. LC_{50} calculated using nominal exposure concentrations.

more than 3 orders of magnitude lower, however, than the LC_{50} determined for adults of the same stock in time-toxicity studies (see Section 3.1). Whether this difference in toxicity thresholds reflects differences in fish age, size, health, experimental conditions (specifically partial-replacement versus flow-through conditions) or changes in methoxychlor resistance cannot be determined from the present studies.

3.4 Hatching Studies

In hatching studies with rainbow trout, eyed eggs (age approximately one week prehatch) and alevins (age 3 d posthatch) were exposed to a 2-h pulse of Sanex-methoxychlor 25E. The test alevins included both fish that had been exposed to methoxychlor as eggs and naive fish (not previously exposed). The results are summarized in Tables 7 and 8.

Table 7. Effects of methoxychlor on hatching and survival of rainbow trout, when the fish were exposed to a 2-h pulse as eggs approximately one week prehatch. The entries are means with 95% confidence limits (n = 5 groups of 100 eggs each).

Nominal methoxychlor concentration (µg/L)	Measured methoxychlor concentration (µg/L)[a]	Total hatch (%)	Time of median hatch[b] (days postexposure)	Duration of hatch[c] (days)	18-day posthatch alevin survival (%)
0	<0.03	96 ± 2	5.2 ± 0.1	4.4 ± 1.3	96 ± 2
150	46	98 ± 2	5.1 ± 0.2	3.8 ± 0.4	95 ± 2
300	120	96 ± 2	5.1 ± 0.2	3.8 ± 1.0	95 ± 1
600	270	99 ± 1	5.0 ± 0.1	3.1 ± 0.7	94 ± 3
1,200	580	97 ± 3	5.0 ± 0.2	4.2 ± 0.9	94 ± 2

[a]At the end of the 2-h pulse.
[b]Calculated by linear interpolation.
[c]Calculated between 2.5 and 97.5 percentile of total hatch.

Table 8. Lethality of a 2-h pulse of methoxychlor to 3-day-old alevins of rainbow trout, as a function of their history of methoxychlor exposure.

Nominal methoxychlor concentration (µg/L)	Measured methoxychlor concentration (µg/L)[a]	15-day survival (%)		
		Naive fish[b]	Previously exposed as eggs to (µg/L)	
			0[c]	120[c]
0	<0.06	96 ± 2	100	100
150	27	94 ± 4	100	100
300	40	90 ± 4	100	90
600	203	93 ± 3	100	100
1,200	402	92 ± 7	100	100

[a]At the end of the 2-h pulse.
[b]n = 5 groups of 100 alevins each. Mean presented with 95% CL.
[c]n = 10 fish.

Hatching in fish is not a developmental event per se; fish hatch at different developmental stages dependent primarily upon environmental conditions. Hatching requires the synchrony of a number of physiological and behavioural processes, including secretion of the hatching enzyme by glands near the head and appropriate embryonic movements to distribute the enzyme throughout the perivitelline space. Hatching tends to be a sensitive indicator, therefore, of environmental stressors (Rosenthal and Alderdice, 1976).

Pulsed exposure of eggs to ≤580 µg/L methoxychlor had no detectable impact on egg survival, hatchability, the timing of hatch (time of 50% hatch and duration of 95% hatch), or subsequent hatchling (alevin) survival (Table 7). This is consistent with the 2-h pulsed threshold for sublethal effects to flagfish eggs (1,300 µg/L, Holdway and Dixon, 1984) and the 96-h LC_{50} for brook trout eyed eggs (>50,000 µg/L, Johnson and Finley, 1980).

Similarly, 2-h exposure of 3-day-old alevins to ≤402 µg/L methoxychlor did not significantly affect survival, independent of whether or not the alevins had been previously exposed to methoxychlor as eggs (Table 8). This is in keeping with the 96-h LC_{50} for brook trout alevins (>400 µg/L, Johnson and Finley, 1980). It is possible, nonetheless, that test alevins were impacted sublethally. The 2-h pulsed threshold for sublethal effects to young flagfish (8 d old posthatch) is 250 µg/L (Holdway and Dixon, 1986). In light of the methoxychlor concentration in the Athabasca River following treatment (Figure 3), however, it is unlikely that embryonic or larval

fishes in the Athabasca River, outside the immediate downstream mixing zone, experience methoxychlor concentrations as high as 250 µg/L.

3.5 Studies of Embryonic and Alevin Development

Analytical constraints prevented a thorough examination of the methoxychlor analyses of water. One would expect, however, that the exposure regimes were similar to those documented in Part V and summarized in Figure 3.

Viability of the eggs used in this study was low. Histological examination of 50 eggs collected at experimental time zero (egg age, 1.5 d postfertilization) indicated that only 64% of the eggs were viable; there was no evidence that the remainder had been fertilized. Subsequent calculations of egg survival were corrected to take this into account.

At experimental time zero, the rainbow trout eggs weighed (mean ± 95% CL) 34.7 ± 2.7 mg, of which 1.0 ± 0.1 mg was dry egg capsule, 2.2 ± 0.6 mg perivitelline fluid, 11.4 ± 1.1 mg dry yolk, and 14.7 ± 1.5 mg imbibed water; embryo tissue weight at this time was >0.1 mg. Subsequent weight changes in each of these biotic components are summarized in Figure 4. A general explanation of the patterns observed during early fish development can be found in Heming and Buddington (1987). Briefly, as development proceeds, yolk is absorbed and converted into tissues. The conversion is less than 100% efficient and, consequently, there is a continual loss of bulk dry matter (yolk plus tissues) as some of the absorbed yolk nutrients

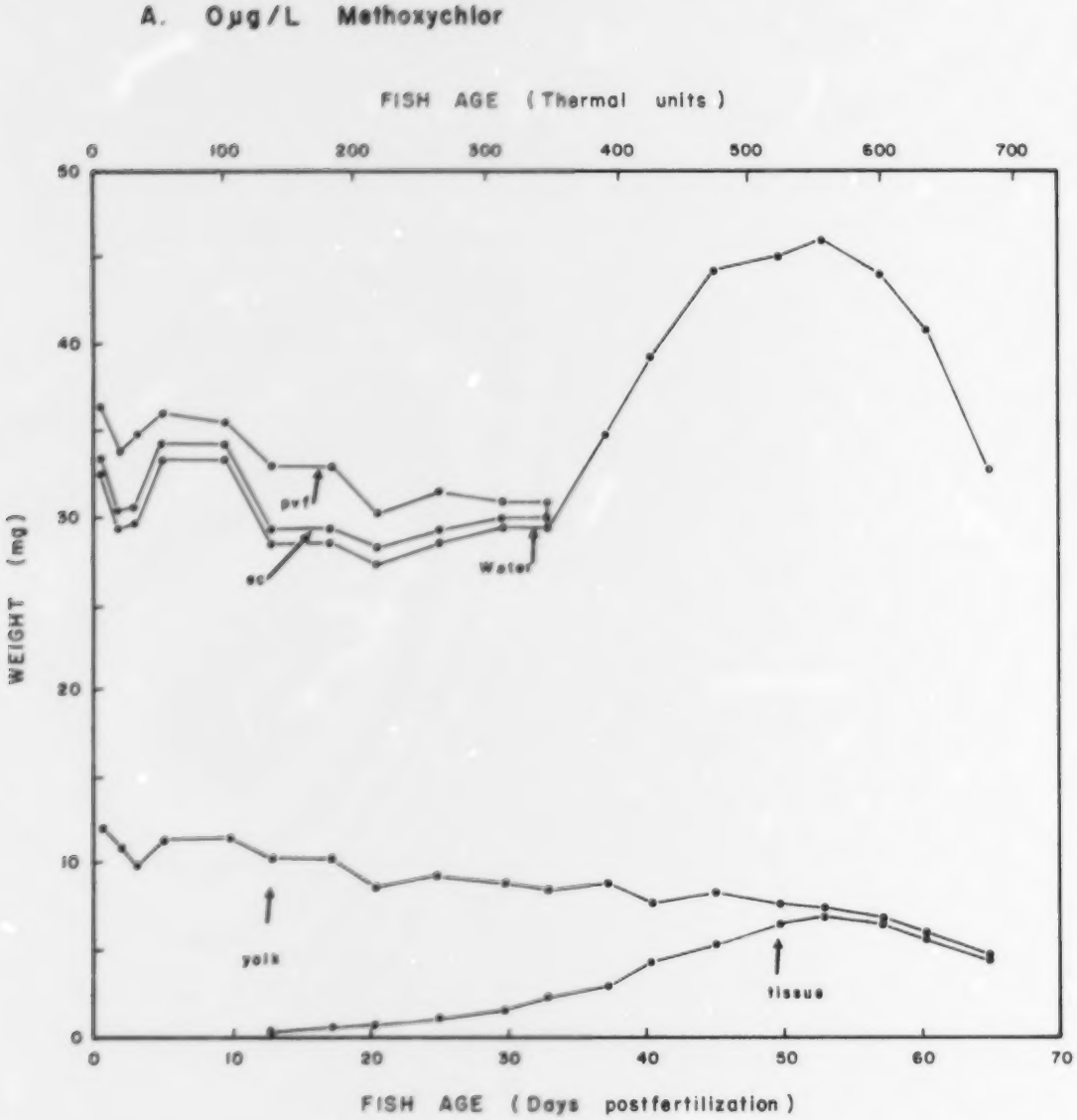

Figure 4. Temporal cumulative changes in the composition of rainbow trout eggs and alevins, when the fish were exposed as eggs to a spike of methoxychlor at 1.5 d postfertilization. Data points are mean values (n = 5.16). Physico-chemical characteristics of the test solutions (means with ranges) were: temperature 10.1°C (8.0- 15.8), pH 7.8 (7.2-8.4), dissolved oxygen concentration 10.4 mg/L (9.7-11.2), conductivity 333 µsiemens/cm (293-423). ec = egg capsule; pvf = perivitelline fluid.

B. 3 µg/L Methoxychlor

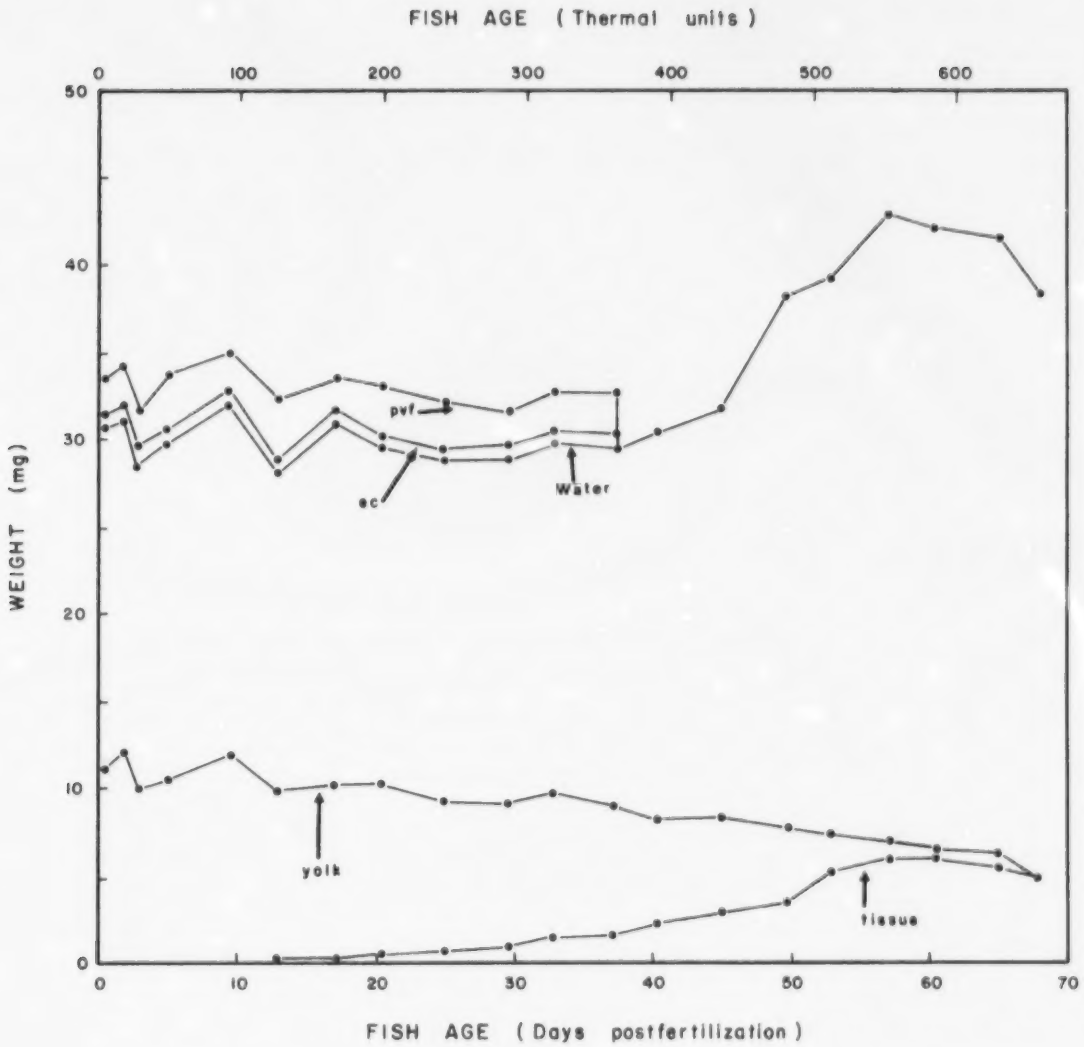

Figure 4. Continued.

C. 30 µg / L Methoxychlor

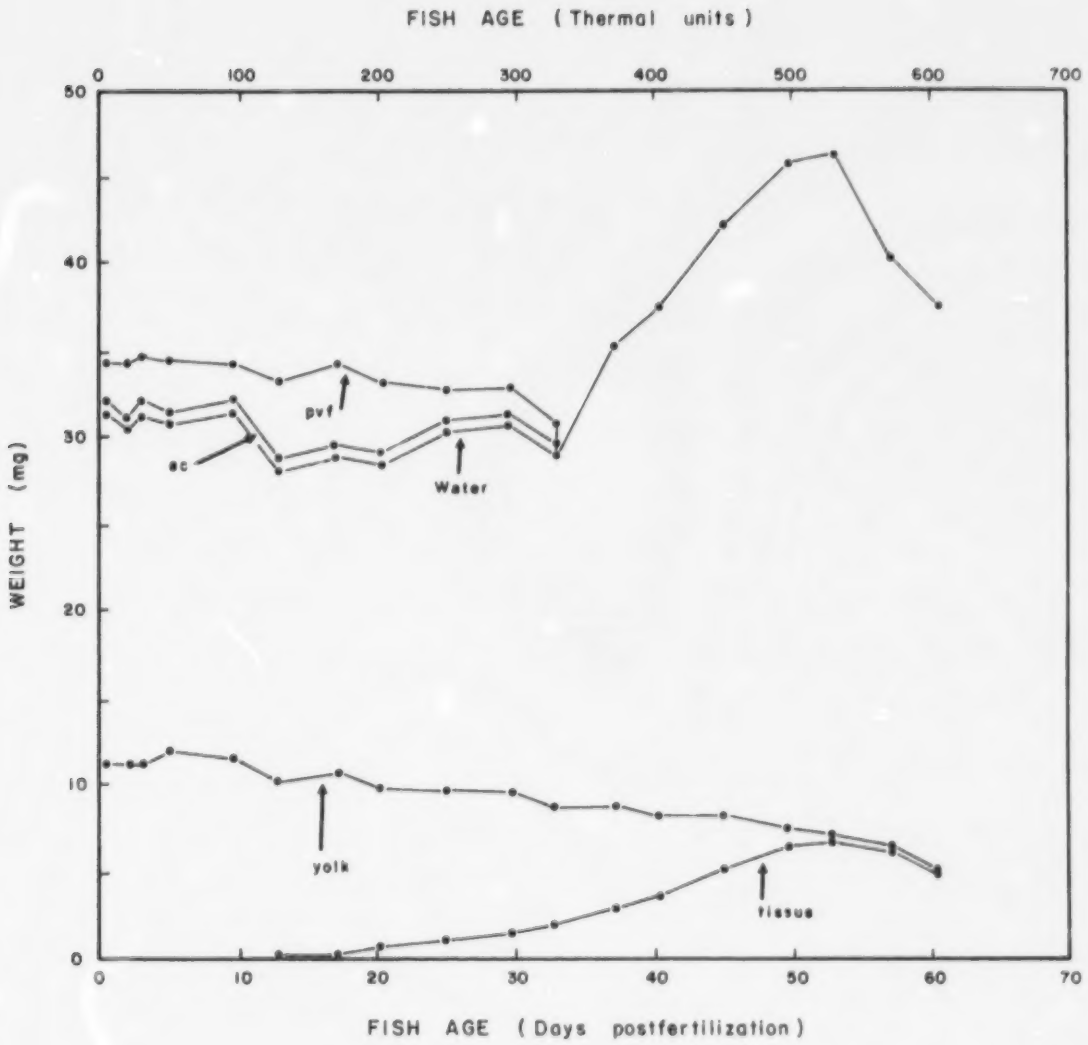

Figure 4. Continued.

are used to provide metabolic energy. Fish yolk tends to be much denser than fish tissues (in the present study; percent water content, yolk = 56 ± 1%, tissues = 81 ± 2%) and hence, as yolk is converted into tissues the amount of imbibed water increases. This is reflected by increases in total wet weight. In the absence of an exogenous food supply, the alevins eventually incur a metabolic deficit after which simple yolk absorption is insufficient to satisfy all metabolic demands and tissues begin to be catabolized. At this point, tissue dry weight begins to decline although yolk reserves are still present.

Yolk absorption and utilization are sensitive indicators of some environmental stressors (Heming and Buddington, 1987). Under the present experimental conditions, methoxychlor had no detectable effect on the absorption or utilization of yolk by rainbow trout (Table 9). There were no significant differences among treatment groups ($P>0.05$) with respect to the rate of yolk absorption, the rate of tissue elaboration (growth), the gross conversion efficiency of yolk to tissues, or the maximum tissue weight achieved on yolk alone. In each treatment group, the maximum tissue weight was attained at the same stage of development (Vernier's stage 35 and $k_D = 4.2$).

The effects of a spiked exposure to methoxychlor on hatching behaviour and success are summarized in Table 10. In the present studies, methoxychlor would appear to have little or no effect on egg survival (hatchability) or on subsequent hatchling (alevin) survival. Exposure of freshly fertilized eggs to a nominal spike of 30 µg/L methoxychlor, however, did impact hatching behaviour. At the highest

spiked concentration, fish hatched significantly earlier and more rapidly than control fish. Premature hatching is a characteristic response to some environmental stressors (Rosenthal and Alderdice, 1976). In the present case, these behavioural effects were probably of minimal biological significance, given that the timing of 50% hatch was shifted by less than one day. Further work would be required to determine whether or not the underlying neurological/physiological causes of the changes in behaviour have significant impacts on later survival.

Table 9. Effects of methoxychlor on yolk absorption and utilization in rainbow trout, when the fish were exposed to a spike of methoxychlor at 1.5 d postfertilization.

Nominal spiked methoxychlor concentration (µg/L)	Yolk absorption rate[a] (mg/tu)	Specific tissue growth rate[a] (%/tu)	Gross yolk conversion efficiency[b] (%)	Maximum tissue dry weight[c] (mg)
0	0.030 (0.025-0.035)	0.62 (0.47-0.76)	57	6.8 ± 0.4
3	0.032 (0.028-0.036)	0.64 (0.53-0.75)	55	6.0 ± 0.5
30	0.033 (0.029-0.037)	0.62 (0.49-0.75)	60	6.7 ± 0.3

[a]Calculated between hatching and attainment of maximum tissue weight. Presented with 95% CL.
[b]Calculated from fertilization to attainment of maximum tissue weight.
[c]n = 15. Presented with 95% CL.
tu = thermal unit or degree-day.

Table 10. Effects of methoxychlor on hatching and survival of rainbow trout, when the fish were exposed to a spike of methoxychlor at 1.5 d postfertilization.[a]

Nominal spiked methoxychlor concentration (µg/L)	Total hatch (%)	Time of median hatch[b]		Duration of hatch[d]		30-day posthatch alevin survival (%)
		tu	days[c]	tu	days[c]	
0	51	343 (340-345)	34.0 (33.7-34.2)	38	3.8	91
3	50	344 (342-346)	34.1 (34.0-34.3)	30	3.0	95
30	48	336 (335-337)	33.3 (33.2-33.4)	21	2.1	92

[a]n = 1 group of 1066 eggs at 0 µg/L, 1087 eggs at 3 µg/L, and 1093 eggs at 30 µg/L.
[b]Calculated by interpolation. Presented with 95% CL.
[c]At 10.1°C.
[d]Calculated between 2.5 and 97.5 percentile of total hatch.
tu = thermal units.

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PART V

HISTOPATHOLOGICAL EFFECTS ON RAINBOW TROUT OF LONG-TERM EXPOSURE TO METHOXYCHLOR

1. INTRODUCTION

The purpose of this chapter is to describe the effects of long-term exposure to industrial-grade methoxychlor on the structure of tissues of rainbow trout. Additional studies were conducted on the effects induced by the emulsifier, reagent-grade methoxychlor and the solvent used in experimental studies.

2. MATERIALS AND METHODS

All experimental fish were obtained from stocks as outlined in Part IV (p. 24). Exposures were conducted using rainbow trout measuring 5.9 cm (range 3.8-8.9) in fork length and weighing 2.4 g (range 0.5-8.2). All tests were conducted in 40-L tanks containing dechlorinated municipal water. Temperatures were maintained at an average 10.7°C (range, 10.5-10.9); dissolved oxygen averaged 10.0 mg/L (range, 9.4-10.5); pH averaged 7.6 (range 7.5-7.7), and conductivity averaged 351 µS/cm (range 348-355). Other water quality parameters are reported in Part IV (Table 1).

The exposure regimes consisted of a control using dechlorinated water, ethanol plus dechlorinated water, three concentrations of reagent-grade methoxychlor plus ethanol, three concentrations of the emulsifier used in the preparation of industrial-grade methoxychlor, and three concentrations of industrial-grade methoxychlor (Table 1). The ethanol was used as a solvent for reagent-grade methoxychlor to facilitate solubilization in the experimental waters. The water in each tank was partially (50%) replaced each day, except on weekends.

All tanks were lightly aerated through the exposures, except during replacement.

Reagent-grade methoxychlor or industrial-grade methoxychlor, and the emulsifier, were added to the experimental tanks from stock solutions. Three stock solutions were prepared:

a. Reagent-grade methoxychlor (500 mg/L) dissolved in 95% ethanol.

b. Industrial-grade methoxychlor (1% solution).

c. Emulsifier (5000 mg/L).

The ethanol content of each test solution including the control and ethanol was adjusted to 3.84 ml, equivalent to a final concentration of 157 mg/L ethanol in each tank.

Table 1. Exposure regimes used in this study.

I.	Control (Dechlorinated Municipal Water)
II.	Ethanol and Dechlorinated Water
III.	10 µg/L Reagent-Grade Methoxychlor + Ethanol
IV.	20 µg/L Reagent-Grade Methoxychlor + Ethanol
V.	40 µg/L Reagent-Grade Methoxychlor + Ethanol
VI.	30 µg/L Emulsifier
VII.	60 µg/L Emulsifier
VIII.	120 µg/L Emulsifier
IX.	10 µg/L Industrial-Grade Methoxychlor
X.	20 µg/L Industrial-Grade Methoxychlor
XI.	40 µg/L Industrial-Grade Methoxychlor

Due to analytical restraints, it was not possible to determine the actual concentration of methoxychlor, emulsifier or ethanol in any tank. It is likely that the nominal concentrations of these agents were higher than the actual exposure concentrations, reflecting loss through volatilization and sorption.

A total of 55 fish was used in each exposure regime. Fish were fed twice during the first week of exposure but, because the tanks became excessively dirty, feeding was reduced to once per week for the remainder of the experiments. Fish were fed a commercial mixture (Sterling Cup #2 fry) of one day's ration (3.8% of body weight) on each feeding date. This constituted a maintenance diet only.

A sample of approximately 10 fish was taken at the start of each experiment (time zero) and at day 7, 14, 21 and 28. Each fish was slit open at the abdomen and immediately placed in Bouin's fixative solution. After 24 h, tissues were preserved in 70% alcohol until trimming.

Tissues were routinely processed and sections of 5 to 6 microns in thickness were prepared and stained with hematoxylin and eosin. Gills, heart, oral cavity, eye, brain, spinal cord, liver, stomach, pyloric ceca, intestine, pancreas, spleen, anterior and posterior kidneys, gonads, skin and muscle were histologically examined in each fish.

Fish livers were also fixed in glutaraldehyde, dehydrated with organic solvents and embedded in Epon. Ultrathin sections were then analyzed by transmission electron microscopy.

During the experimental period, there were no mortalities in the control tanks. Mortality in the other tanks was also either nil or low (Table 2).

Table 2. Mortality of rainbow trout under different experimental regimes.

Regime	Number of dead fish	Time (days)
I. Control	0	--
II. Control + Ethanol	0	--
III. 10 µg/L Methoxychlor + Ethanol	0	--
IV. 20 µg/L Methoxychlor + Ethanol	1	3
V. 40 µg/L Methoxychlor + Ethanol	1	21
	1	28
VI. 30 µg/L Emulsifier	3	3
VII. 60 µg/L Emulsifier	2	3
VIII. 120 µg/L Emulsifier	1	3
IX. 10 µg/L Industrial Methoxychlor	1	3
X. 20 µg/L Industrial Methoxychlor	1	7
XI. 40 µg/L Industrial Methoxychlor	1	3

3. RESULTS AND DISCUSSION

3.1 Light Microscopy

The frequency of gill and hepatic lesions is summarized in Tables 3 and 4. The gill and hepatic lesions were not indicative of methoxychlor or emulsifier toxicity. The incidence of hepatic vacuolation increased during the last 2 weeks of the experiments (Table 4). The reasons for this increase are unknown. No lesions were observed in any other tissue.

3.2 Electron Microscopy

Several liver samples from fish exposed to 40 µg/L methoxychlor and ethanol for 28 d were examined, along with controls. The hepatic vacuolation seen by light microscopy was characterized ultrastructurally by accumulation of fluid in the cytoplasm of the hepatocytes (Figure 1). Very few lipid droplets were seen in the hepatocytes. Lesions indicative of hepatic toxicity were seen in 3 of 4 principal fish. These lesions were characterized by disintegration and lysis of the endoplasmic reticulum in individual hepatocytes. Vesicles and vacuoles of endoplasmic reticulum were also seen. The mitochondria of some of the affected cells were swollen and contained matrix-dense granules (Figure 2). No vascular changes were seen.

Table 3. Incidence of lesions in the gills of fish under different experimental regimes.

Regime	Time, postexposure (days)				
	0	7	14	21	28
I. Control	0/10[a]	1/10	0/10	0/10	0/10
II. Control + Ethanol	0/10	3/10	4/10	0/10	2/15
III. 10 µg/L Methoxychlor + Ethanol	0/10	2/10	0/10	0/10	0/15
IV. 20 µg/L Methoxychlor + Ethanol	0/10	2/10	0/10	0/10	0/14
V. 40 µg/L Methoxychlor + Ethanol	0/10	0/10	0/10	0/10	0/13
VI. 30 µg/L Emulsifier	0/10	0/10	0/10	0/10	0/10
VII. 60 µg/L Emulsifier	0/10	0/10	0/10	0/10	0/9
VIII. 120 µg/L Emulsifier	0/10	1/10	1/10	0/10	0/10
IX. 10 µg/L Industrial Methoxychlor	0/10	0/10	0/10	4/10	0/10
X. 20 µg/L Industrial Methoxychlor	0/10	0/10	0/10	0/10	0/10
XI. 40 µg/L Industrial Methoxychlor	0/10	0/10	0/10	1/10	0/10

[a] Number of fish with lesions/number of fish examined.

Table 4. Incidence of lesions in the livers of fish under different experimental regimes.

Regime	Time, postexposure (days)				
	0	7	14	21	28
I. Control	2/10	2/10	3/10	6/10	8/10
II. Control + Ethanol	3/10	1/10	0/10	5/10	5/15
III. 10 µg/L Methoxychlor + Ethanol	3/10	2/10	3/10	5/10	6/15
IV. 20 µg/L Methoxychlor + Ethanol	2/10	3/10	3/10	4/10	6/14
V. 40 µg/L Methoxychlor + Ethanol	7/10	1/10	5/10	9/10	3/13
VI. 30 µg/L Emulsifier	4/10	2/10	5/10	10/10	9/10
VII. 60 µg/L Emulsifier	3/10	6/10	4/10	5/10	7/9
VIII. 120 µg/L Emulsifier	1/10	2/10	2/10	6/10	8/10
IX. 10 µg/L Industrial Methoxychlor	5/10	3/10	3/10	9/10	8/10
X. 20 µg/L Industrial Methoxychlor	5/10	4/10	3/10	4/10	8/10
XI. 40 µg/L Industrial Methoxychlor	3/10	2/10	2/10	7/10	6/10

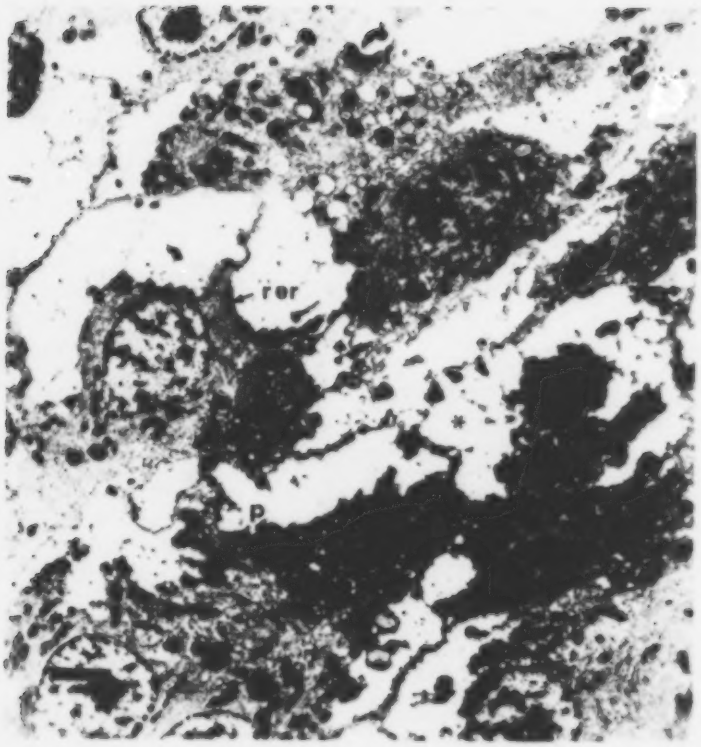

Figure 1. Hepatocytes from a control fish showing normal rough endoplasmic reticulum (rer), perixisomes (p) and displacement of cytoplasm by electron-lucent fluid-filled areas (*). Bar = 3 μm.

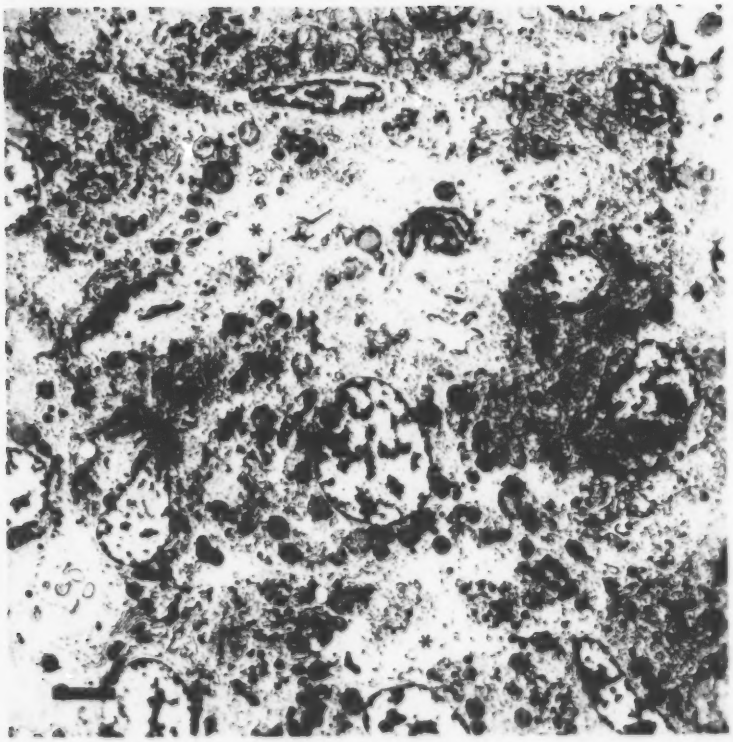

Figure 2. Hepatocytes from a fish exposed to 40 µg/L methoxychlor for 28 d. Note the disintegration and lysis of endoplasmic reticulum (*). Bar = 3 µm.

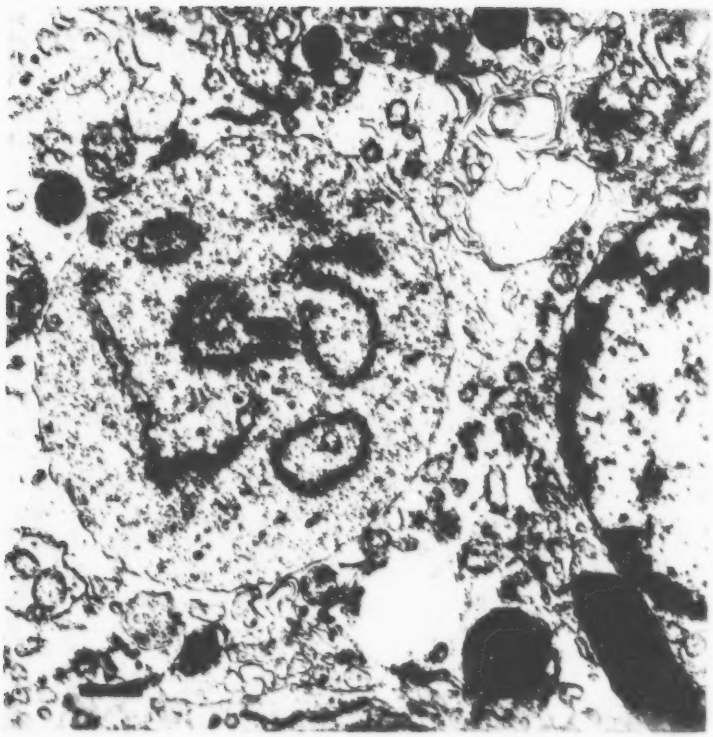

Figure 2a. Higher magnification of an irregular configuration of rough endoplasmic reticulum within a phagosome. Bar = 0.5 µm.

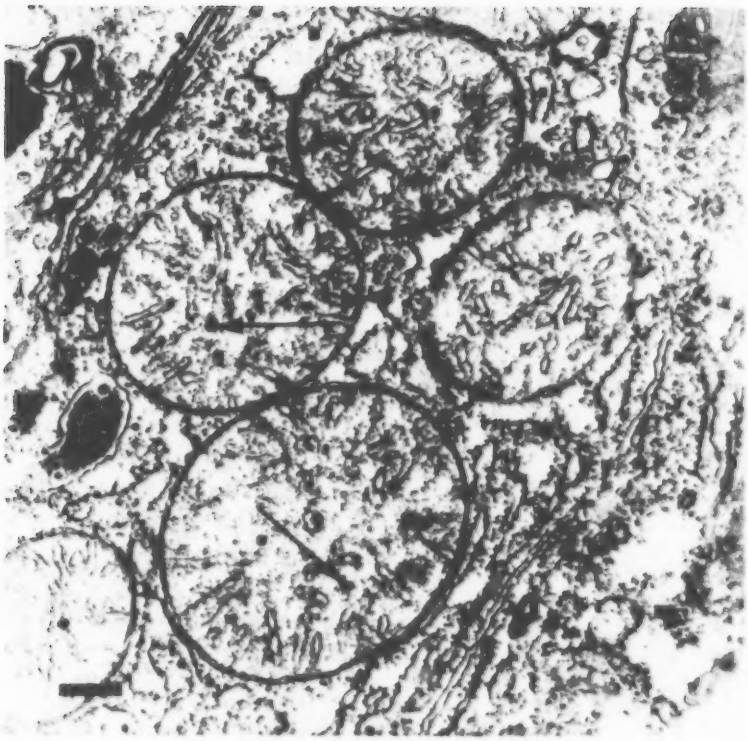

Figure 2b. High magnification of swollen mitochondria with matrical dense granules (arrows). Bar = 0.5 μm.

3.3 Discussion

Histopathological alterations in gills are frequently found in fish exposed to toxic agents. Clubbing of lamellae, necrosis and edema are the most commonly reported gill lesions in fish experimentally exposed to different toxicants, including toxaphene (Lowe, 1964), mirex (Van Valin et al., 1968), heptachlor, formaldehyde, heavy metals, phenols, detergents, and acrylamide flocculants (Abel and Skidmore, 1975; Brown et al., 1968; Mitrovic et al., 1968; Skidmore and Tovel, 1972; Smith and Piper, 1972; Albassam et al., 1987). On the other hand, gill lesions can also be found sporadically in some fish. The type and severity of histological lesions found in the gills of fish exposed to methoxychlor were not indicative of toxicity. In addition, the presence of similar lesions in control and ethanol-treated fish further corroborates that these alterations were unrelated to treatment with methoxychlor.

Damage to the structure of gills is likely to affect gas exchange, movement of anions and cations, and movement of organic substances. Under conditions of severe necrosis, such changes may result in rapid death whereas minor lesions such as those observed in this study may have little or no effect on fish survival under natural conditions.

Liver damage is frequently reported in fish exposed to toxic concentrations of pesticides. Couch (1975), working with spot, found that hepatic lipidosis was the most frequently observed non-specific liver lesion following pesticide exposure. Non-specific liver

degeneration was also reported for rainbow trout exposed to methoxychlor (Walsh and Ribelin, 1975), whereas hepatocyte shrinkage and granulation was observed in bluegills, Lepomis macrochirus, exposed to methoxychlor (Kennedy et al., 1979). Lesions in the liver of the experimental fish in this study were probably not related to methoxychlor exposure, given the fact that similar lesions were observed in control fish. In several cases, the frequency of lesions increased with time, indicating that the experimental regime such as feeding or water quality may have exacerbated the condition.

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